SAPIENZA
UNIVERSITÀ DI ROMA

DOTTORATO DI RICERCA
BIOLOGIA UMANA E GENETICA MEDICA

XXIX CICLO

FETAL SHORT FEMUR LENGTH AS A MINOR MARKER FOR FETAL ANEUPLOIDIES, SKELETAL DYSPLASIA AND INTRAUTERINE GROWTH RESTRICTION: RISK STRATIFICATION FOR ISOLATED AND NOT ISOLATED FINDING IN DIFFERENT GESTATIONAL AGE

Relatore
Prof.Antonio Pizzuti
Correlatore
Prof.ssa Antonella Giancotti

Dottoranda
Dott.ssa Valentina D'Ambrosio
Matricola 694279

Anno Accademico 2016/2017

INDEX

EXPERIMENTAL STUDY

ABSTRACT

Introduction. Fetal short femur is defined by a femur length below the 5th percentile or -2 DS for the gestational age. The finding of a short femur represents a diagnostic dilemma for the various differential diagnosis. It may be associated with skeletal dysplasia, aneuploidies or genetic syndromes. In the isolated form, it may be an early sign of placental insufficiency and growth delay, or a normal variant in constitutionally small fetuses.

Aims of the study: The aim of this study was: to examine postnatal outcome of pregnancies complicated by a short femur length; to compare outcomes in pregnancies with an early diagnosis of short FL (< 24 weeks of gestation) with pregnancies where this sign arises later in gestation (> 25 weeks of gestation); to analyse outcome differences in isolated and non-isolated form. A secondary aim of our research was a proposal of a diagnostic algorithm as a tool to guide clinicians in the management and counselling of pregnancy with isolated and not isolated short femur length. For this purpose, a revision of current literature data on the argument was carried out.

Materials and Methods: A longitudinal prospective cohort study was conducted. All singleton pregnancies with a diagnosis of fetal femur < 5 centile were enrolled in the study. Patients were divided into two groups: patients with diagnosis of FL < 5th percentile at 14-24 weeks (group A) and at 25-40 weeks (group B). The differences in pregnancy complications and outcomes between the two groups were analysed. A comparison of the results of isolated and non-isolated forms was also carried out. For the secondary aim of our study we reviewed the literature and used meta-analytic technique to estimate accuracy of this marker in the prediction of Down Syndrome, IUGR and skeletal dysplasia. Correlation with poor perinatal outcome was also evaluated.

Results: We enrolled 147 cases of short femur length in singleton pregnancies. In 61 (41,49%) cases short femur was associated to other fetal anomalies, in 86/147 fetuses (58,5%) was classified as isolated. Abnormal fetal karyotype (27,3% vs 3.7% p: 0.02) and skeletal dysplasia (19,7% vs 3.7% p: 0.002) were more frequent in group A. Cases of multiple abnormalities was diagnosed in 9 cases in group A and in 6 cases in group B with a difference not statistically significant (13.6% vs 7.4% p < 0.193). Diagnosis of isolated short femur was more common in group B (79% vs 33,4%, p: 0.000). In group B diagnosis of IUGR was made in 44.4% vs 19.7% of group A (p:0.002). The SGA prevalence had a difference statistically significant between the two groups (7.6% vs 24.7% p:0.007). The percentage of live birth was significant lower than group B (34.8% vs 97,6%). A comparison based on presence of an isolated short femur and not isolate finding (Group 1: Isolated - Group 2 not isolated) was also carried out. Abnormal fetal karyotype and (24,6% vs 7,0% p: 0.004), skeletal dysplasia (24,6% vs 1.2% p: 0.004) were more frequent in non-isolated group. Diagnosis of IUGR and SGA was more common in isolated group (47,7% vs 13,1%, p: 0.000, 25,6% vs 4,9% p 0.001) (table 4). The percentage of live birth was significant lower in not isolated group (45.9% vs 86% p 0.00). A higher incidence of neonatal complication, postnatal surgery and neonatal death were notice in not isolated group compared to isolated (57,69% vs17.45% p 0.019; 27,92% vs 4,2% p:0.003).

Meta-analysis showed a higher incidence of short femur length in Down Syndrome fetuses (375/1326 28,2%) compared with euploid group (5809/188935, 3.07%) with an OR 5.12 (95% CI, 4.47-5.87). A higher incidence of IUGR/SGA was found in isolated short femur (455/3108, 14,6%) compared with the control group (11634/222362, 5.23%) with an OR of 4.12 (CI 95% 3.70-4.58).

Conclusions. The diagnosis of short FL is often a challenge in obstetrics. The results of our study could help clinicians in counseling these patients in presence of this ultrasound findings. The diagnosis of a non-isolated short femur length before 24 weeks of gestation is associated to poor pregnancy outcome. When a short femur arises late in gestation and in isolated form, pregnancy outcome is better in term of chromosomal abnormalities but high rate of IUGR, SGA and neonatal complication is possible.

INTRODUCTION

1. PRENATAL DIAGNOSIS: STATE OF ART

Congenital anomalies affect approximately 2% of liveborns but have a major impact on pregnancy loss as well as on perinatal mortality and morbidity [1]. In particular EUROCAT recorded a total prevalence of major congenital anomalies of 23.9 per 1,000 births for 2003-2007. 80% were live births. 2.5% of live births with congenital anomaly died in the first week of life. 2.0% were stillbirths or fetal deaths from 20 weeks gestation. 17.6% of all cases were terminations of pregnancy following prenatal diagnosis [2]. Scientists have been intrigued with congenital malformations since early history, so that many studies were conducted to understand causes, patterns and risk factors. Still today about 60% of congenital anomalies causes in humans remains unknown, however it is recognized that genetics plays a central role in the mechanism of birth defects both as unique cause than in association with environmental risk factors [3].

1.1 Ultrasound in prenatal diagnosis

The use of ultrasonography for obstetrics was developed in the late 1950s as A-mode or amplitude mode ultrasound. A single high frequency sound wave was transmitted and the reflected signal was used to plot the distance and localize the fetal head. In the 3 1970s, B-mode, or brightness-mode was developed using a digital scan converter and black/white images over a contrasting back ground allowed the viewing of static fetal images. Steady development of ultrasonography brought real-time imaging in the 1980s [4,5]. Over the last decades, ultrasonography has become a vital part of obstetric care.

There have been remarkable advances in technology, sonographic instrumentation and in sonographers abilities, so that ultrasound imaging is now considered the key to the prenatal diagnosis of most fetal malformations [6]. This technique is widely available, easy to apply, cost-effective and safe for the fetus. Taking advantage of real-time imaging, ultrasound allows an examination of the external and internal anatomy of the fetus and the detection of not only major congenital anomalies but also subtle markers of chromosomal abnormalities and genetic syndromes. Next to two - dimensional ultrasound (2D) imaging, three - dimensional (3D) and increasingly four - dimensional (4D) ultrasound (includes fetal movements) is being applied in fetal diagnosis. These modalities, through volume acquisition, allow to study anatomical structures of interest in different planes of section. In this way more detailed images of various fetal structures can be obtained [7]. Guidelines regarding obstetric ultrasonography have been published by numerous organizations [8-10]. This prenatal investigation is offered to pregnant women at the optimal gestations age during pregnancy, by public health system in mostly national and international screening programs.

1.1.1 First trimester ultrasound examination (before 14 week of gestation)

The first trimester is defined as the first 13 weeks + 6 days of pregnancy following the last normal menstrual period. It can be divided into three phases, each of which has typical clinical issues: conception phase (3-5 week); embryonic phase (6-10 weeks) fetal phase: (10-12 weeks). During the first trimester, the pregnancy progresses from a tiny gestational sac with no visible embryo, to a 84 mm fetus with identifiable features and internal organs. In developed countries routine scanning is offered during first trimester to assess early fetal development. Current guidelines recommend to perform

this scan before 14 weeks of gestation to confirm viability, accurately establish gestational age, determine the number of fetuses and, in the presence of multiple gestation, assess chorionicity and amnionicity. Towards the end of the first trimester, the scan also offers an opportunity to detect major fetal abnormalities and, in health systems that offer first-trimester aneuploidy screening, to measure the nuchal translucency thickness (NT) [9]. NT is the accumulation or collection of fluid behind the fetal neck that is detected during sonographic evaluation in the first trimester 11–13+6 weeks of gestation when Crown-rump-length (CRL) of the fetus measures from 45 to 84 mm. An increased NT has been associated with trisomy 21, as well as other chromosomal abnormalities, genetic syndromes, and structural malformations [11]. In early pregnancy, it is possible to recognize with confidence certain types of fetal malformations, like anencephaly, which can be reliably diagnosed at 10-14 weeks of pregnancy. In some cases omphalocele and limb anomalies are also definable using ultrasound in the first trimester, while other structural malformations, like urinary tract abnormalities, are detectable later in pregnancy. Detection rates (DRs) of first-trimester fetal anomalies ranged from 32% in low-risk groups to more than 60% in high-risk groups, demonstrating that first-trimester ultrasound has the potential to identify a large proportion of fetuses affected with structural anomalies. The use of a standardized anatomical protocol improves the sensitivity of first trimester ultrasound screening for all anomalies and major anomalies in populations of varying risk [12]. (Figure 1.)

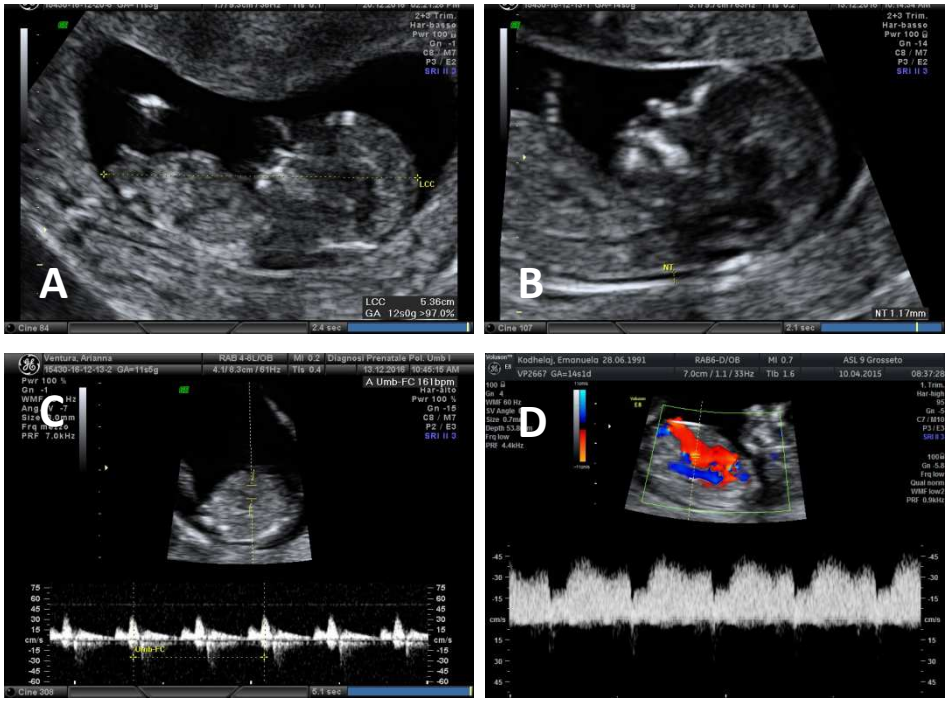


Figure 1. Sonograms showing first trimester fetal ultrasound examination : (a) CRL; (b) NT and Nasal Bone; (c) Tricuspid Flow, (d) Ductus venosus flow.

1.1.2 Second trimester ultrasound examination: anomaly scan (19-22 weeks of gestation)

The second trimester is an important time period for prenatal screening. This is the optimal gestational age to screen for fetal structural anomalies. Mid-trimester scan is also used to check fetal growth by fetal biometry of head, abdomen and long limbs and to date pregnancy for patients that have missed the first trimester screening period. Anomaly scan should be offered routinely to all patients, preferably between 18 and 22 weeks of gestation by an appropriately trained sonographer and with equipment of an appropriate standard. Prenatal screening examination includes an evaluation of the following: cardiac activity; fetal number (and chorionicity if multiple pregnancy); fetal age/size; basic fetal anatomy; placental appearance and location. The performance of mid-trimester ultrasound seems better if an scan protocol is used (Table a)[10]. The accuracy in detecting malformations by ultrasound, however, shows great variability

among centers and operators. Large studies and reviews report DRs of 16–44% of anomalies in the second trimester, with higher DRs (84%) of major and lethal anomalies [13,14]. EUROCAT registers report a DR of about 40% [15]. (Figure 2)

Table a. Recommended minimum requirements for basic mid-trimester fetal anatomical survey according ISUOG guidelines (10)	
Head	Intact cranium Cavum septi pellucidi Midline falx Thalami Cerebral ventricles Cerebellum Cisterna magna
Face	Both orbits present Median facial profile Mouth present Upper lip intact
Neck	Absence of masses (e.g. cystic hygroma)
Chest	Heart Normal appearing shape/size of chest and lungs Heart activity present Four-chamber view of heart in normal position Aortic and pulmonary outflow tracts No evidence of diaphragmatic hernia Three-vessel cord
Abdomen	Stomach in normal position Bowel not dilated Both kidneys present Cord insertion site Genitalia Male or female
Skeletal	No spinal defects or masses (transverse and sagittal views) Arms and hands present, normal relationships Legs and feet present, normal relationships
Placenta	Position No masses present Accessory lobe Umbilical cord

1.1.3 Third trimester ultrasound (28-32 weeks of gestation)

The purpose of third trimester ultrasound assessment is the evaluation of fetal growth and the check of amniotic fluid index and placenta insertion. Full evaluation should include assessment of fetal cardiac activity, fetal presentation and lie, measurements of fetal size, placental localization, amniotic fluid volume. Fetal weight is esteemed from biometric parameters and usually compared with Hadlock fetal growth charts to exclude intrauterine growth restriction (IUGR) or large for gestational age fetuses (LGA). In fetuses at risk for growth pathologies an assessment of fetal Doppler ultrasound is required for wellbeing. This consist in a registration of pulsatility index in Umbilical artery and in middle cerebral vascular artery to exclude impaired fetal oxygenation. Even if the purpose of third trimester ultrasound in not the exclusion of anomalies, fetal anatomy must be checked. Italian guidelines recommend the visualization of four-chamber-view, stomach, kidneys, bladder and lateral ventricle of fetal head [8]. Policies regarding routine third trimester obstetrical ultrasound differ among countries. In some European countries (France, Switzerland, Belgium, and Germany), it is common practice to include routine third trimester ultrasound as part of normal prenatal care [15]. In Italy the new basic health care levels in force from January 2017, affirm that a third trimester ultrasound should be offered only in high risk pregnancies [16]. So this scan is not recommended in the low-risk pregnancy population. This decision was argued, because many fetal anomalies could not be detectable before the third trimester of pregnancy. In countries that permit late termination of pregnancy (TOP), such as France, a policy of routine third trimester ultrasound is easier to justify. But also if TOP is not permitted, third trimester ultrasound could be lifesaving when a fetal

malformation is diagnosed for the management of pregnancy. The location, timing, and route of delivery may be modified in order to improve the neonatal outcome. [17, 18]

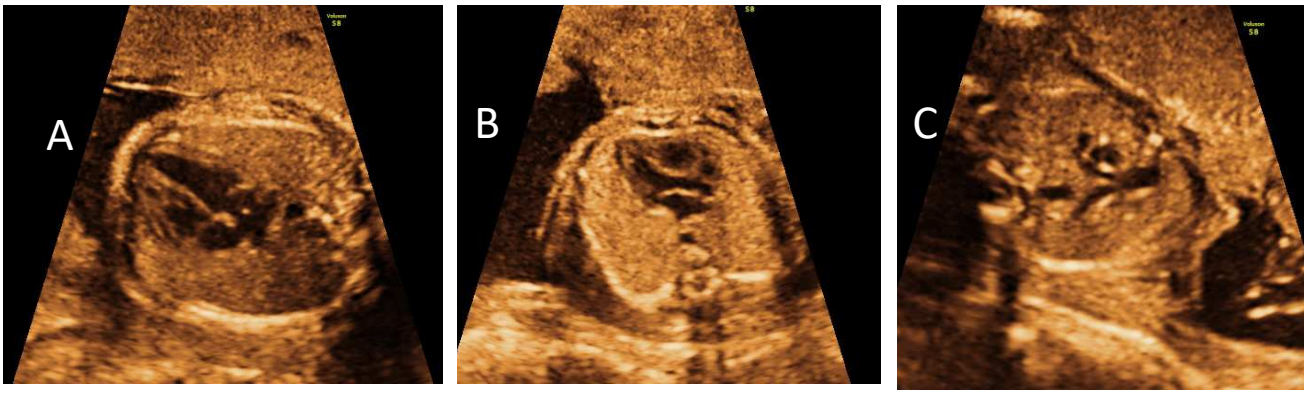


Figure 2 . Sonograms showing mid-trimester ultrasound scan of fetal heart: (a) four-chamber view; (b-c) Aortic and pulmonary outflow tracts

1.2 Non-invasive screening for fetal aneuploidies.

In the last decades, we assisted to remarkable advances in prenatal screening for aneuploidy, particularly in the identification of Down Syndrome [19]. Definitive prenatal diagnosis for chromosome disorders requires invasive sampling followed by karyotype analysis. However, invasive tests are costly and pose a risk of procedure-related complications including miscarriage [20] so that could not be used as a routine in general population. In the 1980s in many developed countries invasive prenatal tests were offered to pregnant women with advanced age (>35 years). This strategy was not efficient because less than one third of Down syndrome pregnancies were diagnosed prenatally and of those undergoing invasive prenatal diagnosis only about 2% had fetal karyotype abnormalities [21]. The introduction of second-trimester serum analyses improved the screening performance for aneuploidy from a 30% of the advanced maternal age to 60-84% with a false positive rate (FPR) of 5% [22, 23]. The proportion

of Down syndrome pregnancies diagnosed was more than doubled and a chromosomal abnormality was found in as many as 4% of those designated as 'screen-positive' [24]. Despite the enhancement, this screening strategy was considered still not satisfactory for the high rate of false negative and the late diagnosis of chromosomal abnormalities that could imply pregnancy termination at an advanced gestational age stressful for the couple. In the early 2000s screening for aneuploidy showed a substantial development with the diffusion of combined test. This test, performed during the first trimester (11-13 weeks+6), consisted in ultrasound measurement of NT together with maternal serum concentration of placental proteins free beta human chorionic gonadotropin (free-ßhCG) and pregnancy-associated plasma protein-A (PAPP-A). This test detects about 90% of fetuses with major aneuploidies with a FPR of about 5% [25]. The performance of combined test can be improved by assessing additional ultrasound markers such as nasal bone, ductus venosus flow and Doppler flow across the tricuspid valve [26-28]. Recently, analysis of cell-free DNA (cfDNA) in maternal blood for non-invasive prenatal testing (NIPT) has been introduced as a method of screening for fetal aneuploidies [29]. The current literature showed that this test is highly accurate in the detection of common fetal autosomal trisomies: maternal blood in singleton pregnancies could detect >99% of fetuses with trisomy 21, 98% of trisomy 18 and 99% of trisomy 13 at a combined FPR of 0.13%. The testing is also routinely offered for the detection of sex-chromosome abnormalities although robust estimates for the DRs, FPRs and positive predictive values (PPVs) are not well established for these disorders [30]. Efforts have therefore been made to extend NIPT to identify additional imbalances, microdeletion and microduplication. Some companies have launched expanded content including a discrete set of microdeletion syndromes [31, 32]. Despite the superior of this

screening test, at present the cost is too high to be adopted as primary test of screening. Therefore NIPT test could be used in association to first trimester combined screening in a contingent model where first-trimester combined testing is offered to all patients as a triage and assessment of cfDNA as a secondary test in a smaller proportion of pregnancies [33].

1.3 Prenatal invasive procedure

Up till the early 1970s, prenatal diagnosis of congenital anomalies was primarily aimed at detecting chromosomal abnormalities by amniocentesis [34]. At present, invasive prenatal diagnosis continues to be the gold standard for pregnancies at increased risk for chromosomal anomalies or other genetic diseases, for whom such time-consuming procedures are believed to be cost-effective, also accounting for procedure-related abortive risks. Chorionic villus sampling (CVS) is the procedure of choice for the first trimester [35]. CVS technique is performed from 11-13 weeks of gestation. There are two types of CVS procedures: transcervical and transabdominal. In the transvervical CVS a catheter is inserted through the cervix into the placenta to obtain the tissue sample. Transabdominal CVS consists into a sample of chorionic villi by a needle inserted through the abdomen and uterus into the placenta [8, 36]. Amniocentesis continues to be performed in mid-trimester gestation [37]. This technique consists in collecting a sample of amniotic fluid from the uterine cavity using a needle via a transabdominal approach. Both procedures are ultrasound guided. CVS and amniocentesis are offered to pregnant women with an increased chance of a fetal chromosomal or genetic disorder. In particular indications to offer a diagnostic test include:

- ✓ Increased risk of abnormality identified through antenatal screening for aneuploidies (combined screening or NIPT test);
- ✓ Previous pregnancy affected with a chromosomal or genetic condition;
- ✓ Parents known carriers of a genetic condition;
- ✓ Family history of a genetic condition;
- ✓ Ultrasound scan showing fetal abnormalities which are associated with a chromosomal or genetic condition. [8,36]

Historically, the targets of prenatal diagnostic testing for women undergoing invasive testing have been limited to whole chromosome aneuploidy (e.g., trisomy 21, trisomy 13, trisomy 18, and monosomy X), molecular genetic diseases with known cause (Fragile X syndrome, cystic fibrosis, and sickle cell disease), and discrete microdeletion syndromes (e.g., 22q11 deletion syndrome and Cri-du-Chat). Any testing performed beyond the standard karyotype has been limited to those indicated on the basis of family history of phenotype. The availability of chromosomal microarray analysis (CMA) has enabled clinicians to cast a wide net for clinical diagnosis. CMA can simultaneously detect whole chromosome aneuploidy, a wide variety of copy number variants (CNVs), including all known microdeletion/microduplication syndromes, and loss of heterozygosity suggestive of either consanguinity or uniparental disomy. Although not yet utilized routinely in prenatal diagnosis, whole genome sequencing and whole exome sequencing are proving to have a role in research (i.e., gene discovery) and clinically in diagnosing genetic conditions that are difficult to diagnose on the basis of phenotype alone [38].

1.4 Additional imaging techniques

- ✓ Fetal echocardiography is an essential tool for screening fetal cardiac anatomy. It is defined as a detailed sonographic evaluation used to identify and characterize fetal heart anomalies before delivery. This specialized diagnostic procedure is an extension of the "basic" and "extended basic" fetal cardiac screening parameters studied in anomaly scan. Two-dimensional imaging is still the gold standard and commonly used in fetal echocardiography therefore color and pulsed wave Doppler give effort to this diagnostic tool. Fetal echocardiography is commonly performed between 18 and 22 weeks of gestation. Some forms of congenital heart disease may even be recognized during earlier stages of pregnancy so that first-trimester fetal echocardiography is diffusing in specialized fetal medicine center. Congenital heart disease is the most common abnormality in the human fetus, occurring in approximately 8-9 per 1,000 live births. Prenatal diagnosis of cardiac defects is important because it allows families to receive appropriate counseling and to properly prepare for the birth of a child with congenital heart disease [39, 40, 8].
- ✓ Magnetic resonance imaging (MRI) is playing an increasingly important role in the evaluation of fetal genetic disorders and malformations. Although fetal MRI was introduced in the 1980 –90s, the indications for using this technique increased with the introduction of ultrafast T2-weighted sequences. These sequences reduced considerably the acquisition time, obviating the need for fetal immobilization [41]. MRI has the potential to improve diagnostic accuracy of the prenatal imaging. It offers a high spatial, temporal, and contrast resolution, which makes the detailed study of fetal pathologies possible. To date, it has not

been shown that MRI imaging has any adverse effects on the fetal growth or development [42]. MRI is considered as a third diagnostic tool requested only if there is an indication arose during second level ultrasound examination. It is a technique still reserved to tertiary center and it is necessary expertise. Assessment of the fetal central nervous system (CNS) is the major indication for fetal MRI [8].

1.5 Genetic counselling in prenatal diagnosis

Advances in genetics and fetal imaging have improved our ability to secure early prenatal diagnosis of a rapidly enlarging spectrum of genetic and developmental disorders. The complexity of this new information has given rise to a specialized group of medical operators, the genetic counselors, dedicated in helping pregnant patients and couple to understand their genetic risks, cope with the implications of these risks, and use the available genetic technology to improve diagnosis of genetic condition involved in human anomalies [43].

Genetic counseling is a communication process which deals with the human problems associated with the occurrence or risk of occurrence of a genetic disorder in a family. This process involves an attempt by one or more appropriately trained persons to help the individual or family to:

- ✓ comprehend the medical facts including the diagnosis, probable course of the disorder, and the available management,
- ✓ appreciate the way heredity contributes to the disorder and the risk of recurrence in specified relatives,
- ✓ understand the alternative for dealing with the risk of recurrence,

- ✓ choose a course of action which seems to them appropriate in view of their risk, their family goals, and their ethical and religious standards and act in accordance with that decision,
- ✓ to make the best possible adjustment to the disorder in an affected family member and/or to the risk of recurrence of that disorder.

In the prenatal setting, the following represent the most common medical indication for a referral to a genetic counselor:

• Advanced maternal age (maternal age greater than 34 years),

• Positive maternal serum screen,

• Patient or family member with a known mendelian disorder

• Prior pregnancy with a chromosomal disorder,

• Family history of mental retardation or birth defect,

• Fetal anomalies or markers detected by sonogram,

• Recurrent pregnancy loss/stillbirth,

• Infertility,

• Ethnic-based carrier screening,

• Consanguinity,

• Maternal disease and/or teratogen exposure,

• Parental concern [44].

Ultrasound abnormalities could be identified in high risk pregnancy but also in patient with no risk factors. The unexpected finding of an anomaly during routine ultrasound could cause extreme parental concern. Genetic counselors can assist patients by explaining the significance of the finding and the availability of further testing through CVS, amniocentesis, fetal echocardiogram or magnetic resonance. They can also

interpret genetic test results for the patient and aid in the follow-up decisions based on these tests. Interpretation of findings and test results as well as information about any underlying disorder may be critical determinants in helping couples make decisions about the management of their pregnancy [44].

In some situation counseling could be complex: ultrasound markers not clearly correlated with genetic and chromosomal disorders are identified, true anomalies are detected, but it may be unclear if they are associated with an underlying genetic or chromosomal disorder. Family medical history information, maternal serum screening results, and other pertinent information must be gathered to allow for better assessment of genetic risk.

Most genetic counseling in relation to prenatal diagnosis will inevitably, and rightly, be carried out by obstetricians and those involved in primary care, with specialists in medical genetics responsible for those cases where the genetic aspects are complex. Today interdisciplinary fetal medicine groups are growing, where obstetrician, radiologist, medical geneticist, non-medical genetic counselor can meet regularly to discuss specific cases and work together in the management of pregnancy complicated by fetal anomalies.

2. PRENATAL DEVELOPMENT OF HUMAN FEMUR

The femur, or thigh bone, is the longest, heaviest, and strongest bone in the body. It is located in the upper leg. It supports the entire body's weight during most activities. Structurally, the femur is classified as a long bone. It consists of a diaphysis and an epiphysis. The diaphysis is composed by compact bone that surrounds the medullary cavity; epiphyses is located in the expanded ends of the bone and constituted by compact bone exteriorly and spongy bone interiorly, with joint surface covered with hyaline cartilage. Metaphyses are the areas between the epiphysis and diaphysis and include the epiphyseal plate in growing bones. The head of the femur rests inside the acetabulum in the pelvic bone; together, they form the hip joint. The distal end of the femur joins with the tibia and the patella to form the knee joint [1].

2.1 Limb's embryology

The development of the long bones at various gestational ages in the fetus has always been a subject of interest for many clinicians. Skeletal development in the limb starts with formation of limb buds, outgrowths of the lateral body wall. They appear early in the second month of human development as a result of proliferation of mesenchymal cells from the lateral plate mesoderm [2]. At the end of the fourth week of development, limb buds become visible as outpocketings from the ventro-lateral body wall covered by a layer of cuboidal ectoderm, called the apical ectodermal ridge (AER). The forelimbs appear first, followed by the hindlimbs a few days later. AER is an embryonic structure that drives the outgrowth of the limb. It exerts an inductive influence on adjacent mesenchyme, causing it to remain as a population of undifferentiated, rapidly

proliferating cells, the progress zone. Development of the limb proceeds proximodistally. In 6-week-old embryos, the terminal portion of the limb buds becomes flattened to form the hand- and footplates and is separated from the proximal segment by a circular constriction. Later, a second constriction divides the proximal portion into two segments, and the main parts of the extremities can be recognized [3].

During morphogenesis, limbs gradually undergo through the mesenchymal, chondrogenic and osseous phase. Limbs are formed along the three axes:

- proximodistal axis;
- anteroposterior axis;
- dorsoventral axis;

As the limb grows out, mesenchymal cells condense in the center to form the cartilage anlagen of the limb bones. The anlagen develop in a proximal to distal direction, and their development can be described as a series of bifurcations and segmentations that follow an axis along the humerus/femur, through the ulna/tibia and the distal carpal (or tarsal in the foot) anlagen [4]. Patterning along the proximal to distal axis is largely controlled by factors produced by the AER [5]. These include fibroblast growth factors that are important for stimulating proliferation and patterning of the underlying mesenchyme [6-7]. Along the antero-posterior axis, which is responsible for limb development in direction from the thumb to the little finger, cellular interactions are controlled by the cells in the zone of polarizing activity, which expresses sonic hedgehog morphogen. Interactions of dorsoventral axis, which include development of the back of the hands to palms, are primarily controlled by the WNT7 signaling protein [8].

Separation of human digits takes place between the 6th and 8th week of development [3] and is executed by the process of apoptosis in the interdigital spaces [9]. Fingers and toes are formed when cell death in the AER separates this ridge into five parts. Further formation of the digits depends on their continued outgrowth under the influence of the five segments of ridge ectoderm, condensation of the mesenchyme to form cartilaginous digital rays, and the death of intervening tissue between the rays.

Bones in vertebrates are formed via two different processes:

- ✓ intramembranous ossification: bone tissue is directly formed from mesenchymal progenitors, that differentiate in osteoblasts. In the case of long bones in mammals, this process generates the bone collar and sequentially increases its diameter [10];
- ✓ endochondral ossification: mesenchymal cells differentiate into chondrocytes that secrete the characteristic extracellular matrix of hyaline cartilage. Bone is formed by replacing a cartilaginous mold with bone tissues. This is essential for epiphyseal morphogenesis and longitudinal growth of long bones [2] (Figure 3).

Ossification of the long bones begins by the end of the embryonic period. At about 6-8 weeks after conception skeletal element is formed initially as a condensation of precartilage cells in a histologically homogenous population of mesenchymal cells. These cells undergo differentiation and maturation into chondrocytes that form the cartilaginous skeletal precursor of the bones. Soon after, the perichondrium, a membrane that covers the cartilage, appears. Chondrocytes go through further growth and differentiation via the growth plate (ordered layers of several differentiation states of chondrocytes). As more matrix is produced, the chondrocytes in the center of the cartilaginous model grow in size. Subsequently intramembranous ossification of the

perichondrium and endochondral ossification of cartilage occur. This results in their death and the disintegration of the surrounding cartilage. Blood vessels invade the resulting spaces, not only enlarging the cavities but also carrying osteogenic cells with them, many of which will become osteoblasts. These enlarging spaces eventually combine to become the medullary cavity. As the cartilage grows, capillaries penetrate it. This penetration initiates the transformation of the perichondrium into the bone-producing periosteum. Here, the osteoblasts form a periosteal collar of compact bone around the cartilage of the diaphysis. By the 12^{th} week after conception, bone cell development and ossification ramps up and creates the primary ossification center, a region deep in the periosteal collar where ossification begins**.** While these deep changes are occurring, chondrocytes and cartilage continue to grow at the ends of the bone (the future epiphyses), which increases the bone's length at the same time bone is replacing cartilage in the diaphyses. At birth, the diaphysis of the bone is usually completely ossified, but the two ends, the epiphyses, are still cartilaginous. Shortly thereafter, however, ossification centers arise in the epiphyses. Temporarily, a cartilage plate remain between the diaphyseal and epiphyseal ossification centers. This plate, the epiphyseal plate, plays an important role in growth in the length of the bones. Endochondral ossification proceeds on both sides of the plate. Each of these centers of activity is referred to as a secondary ossification center. When the bone has acquired its full length, the epiphyseal plates disappear, and the epiphyses unite with the shaft of the bone [2-3]. Growth of skeletal elements occurs by the combination of two modes; appositional and interstitial growth. The former is growth by cell proliferation and addition of cells in the primordium, and the latter is growth by enlargement of the

volume of substance (chondrocyte hypertrophy and extracellular matrix deposition) [11].

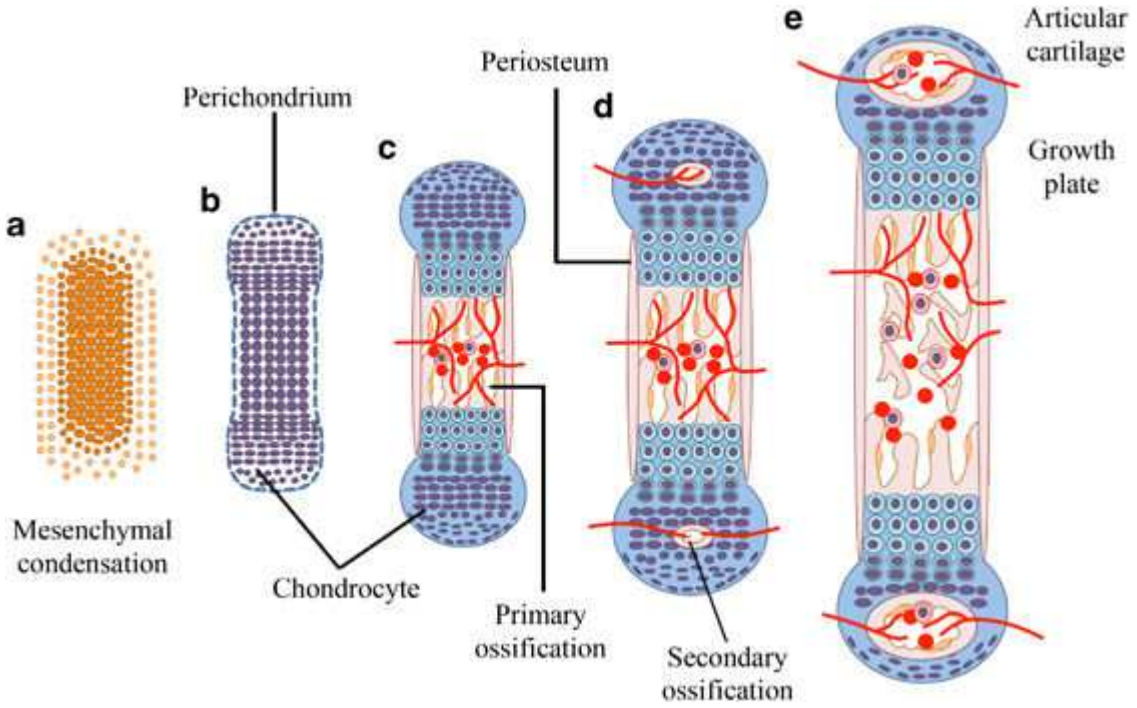


Figure 3 . Endochondral bone formation.

2.2 Regulation of mesenchymal condensation and chondrocyte differentiation in limb skeleton development

The development of cartilage and bone in the primary centers of the skeleton has been the subject of a very large number of researches, and the morphological aspect of the various phases is now known in great detail. The process of intramembranous and endochondral ossification presenting in long bones growth consists in sequential changes of mesenchymal condensations, chondrocyte proliferation and hypertrophic differentiation and terminal replacement with bone. This multistep process is regulated

by a complex network of signaling systems, growth factors and transcription factors [12-13]. The most important of these include sonic hedgehog, secreted by a small group of cells (called the zone of polarizing activity) located at the posterior aspect of the developing limb bud, homeobox transcription factors, and members of the TGF-ß superfamily of signaling molecules [14]. All these molecules are working together as an orchestrated system to generate the normal limb skeletal pattern. Mutations in many of these genes result in striking abnormalities in the limb skeleton.

The choice of mesenchymal cells to differentiate into osteoblasts or chondrocytes is regulated by a canonical Wnt signaling. In areas of membranous ossification, Wnt signaling results in high levels of ß-catenin in mesenchymal cells [15]. This induces the expression of genes that are required for osteoblastic cell differentiation and inhibits transcription of genes needed for chondrocytic differentiation. One of the induced transcription factors, CBFA1/RUNX2 [16-18], in turn induces the expression of another transcription factor called osterix (OSX) [19], and these two factors are critical for the differentiation of mesenchymal cells to osteoblasts. The formation of membranous bones occurs within mesenchymal condensations that are rich in blood vessels. Angiogenesis is an essential part of the process and is controlled by both pro-angiogenic and anti-angiogenic factors. [20]. The differentiation of chondrocytes and formation of cartilage anlagen occur in mesenchymal condensations with low levels of ß-catenin. This results in upregulated expression of the transcription factors of the SOX family. In contrast to membranous bones, vessels are excluded in endochondral ossification. Curiously, the chondrocytes in the anlagen express VEGF-A at a low level [21, 22]. This level of expression is insufficient for stimulating ingrowth of capillaries from the tissue (perichondrium) around the cartilage. However, it is essential for survival of the

proliferating chondrocytes in the end regions (epiphyses) of the developing endochondral bones, and inactivation of VEGF-A expression in chondrocytes at an early developmental stage results in massive chondrocytic cell death in these epiphyseal regions [23]. As development of endochondral bones proceeds, chondrocytes became at the center of the avascular anlagen cease to proliferate hypertrophic and express high levels of the transcription factor CBFA1/RUNX2, and this results in upregulated expression of several genes. Among these genes there is VEGF-A and connective tissue growth factor (Ctgf). The two factors are important for invasion of blood vessels, osteoblastic progenitor cells, and cartilage/bone-resorbing cells from the perichondrium into the hypertrophic cartilage. This invasion results in formation of a primary ossification center, characterized by erosion of the hypertrophic cartilage and its replacement with bone marrow and trabecular bone. The primary ossification center does not form when VEGF-A expression is inactivated, indicating that VEGF-A is crucial for this critical step in endochondral ossification.

The process of mesenchymal condensations, chondrocyte differentiation and proliferation has been shown to be regulated by bone morphogenetic proteins (BMPs) and Sry-box 9. The rate of cartilage differentiation is modulated by parathyroid hormone related peptide (PTHrP) and Indian hedgehog (Ihh), while fibroblast growth factor receptor 3 inhibits proliferation of chondrocytes and promotes hypertrophic differentiation [24-25].

2.3 Biometric evaluation of fetal femur during gestation

Fetal femur length (FL) is defined as the measurement between the distal and proximal ossification centers of the femoral diaphysis. It is a useful parameter in the evaluation of

fetal growth in the second and third trimester of pregnancy. FL is the only long bone measurements required during the routine second trimester scanning by international guidelines. Careful ultrasonographic measurement of only the ossified portions of the diaphysis is needed to obtain accurate measurements. The ossified portion of the femur is measured from the major trochanter to the distal end of the femoral shaft; the distal and proximal epiphyses are not included in the measurement. Oblique planes must be avoided and the femur closer to the transducer must be measured while the transducer is aligned parallel to the long axis of the bone [26, 27]. The sonographic assessment of the FL in utero could be also used for the determination of gestational age with an accuracy of 95% [28]. It is best measured after 14 weeks of pregnancy [29]. It increases linearly throughout pregnancy, as demonstrate in fetal growth chart [30, 31]. Variation in fetal FL is present with respect to maternal race. In particular the fetuses of Asian women have less-than-expected femur lengths and the fetuses of black women have greater-than expected femur lengths than the fetuses of white women in the second trimester [32]. It was supposed that other factors, such as maternal and paternal height, are also important in influencing fetal femur length. On this basis, construction of customized fetal growth charts was produced by Working Group on Fetal Biometric Charts of the Italian ultrasound Society of Obstetrics and Gynecology (SIEOG). A significant relationship between fetal biometric data and parental constitutional characteristics, parity, and race was documented in a large population of low-risk singleton pregnancies. For FL values, paternal and maternal height, maternal weight, and Central or North African maternal race were found to be significant covariates [33]. (Figure 4, 5)

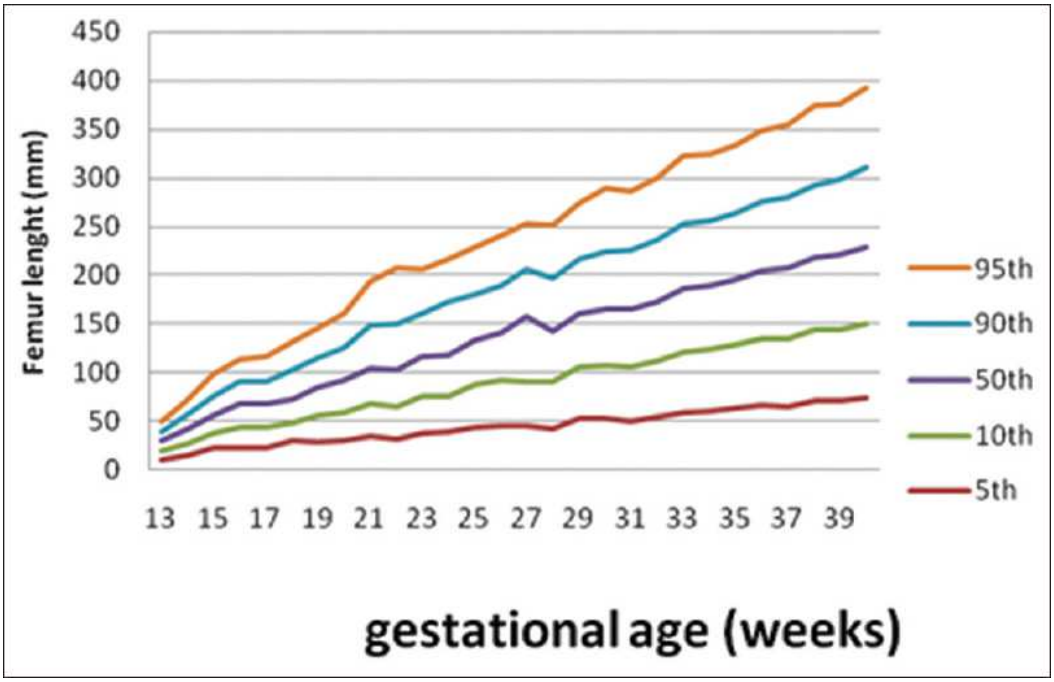


Figure 4 . Femur length biometric Chart

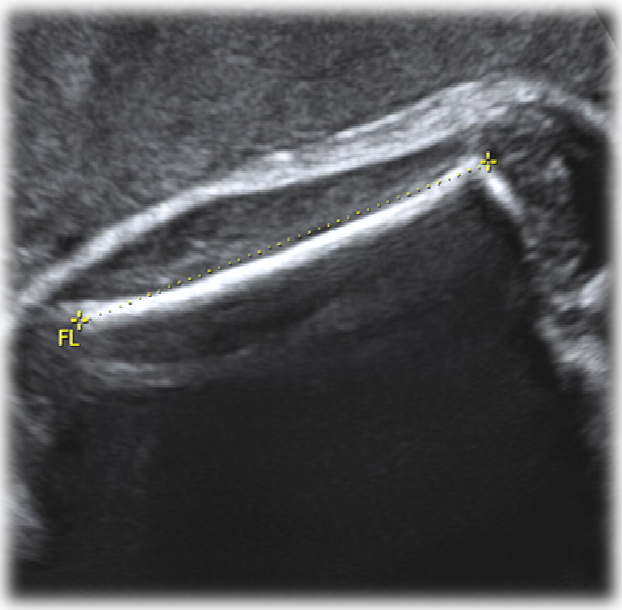


Figure 5 . Sonograms showing femur length measurement

3. FETAL SHORT FEMUR LENGHT: DEFINITION AND IMPLICATIONS

A short FL has been defined as the presence of biometric value of fetal femur below the 5th percentile or -2 standard deviations (SDs) for gestational age at ultrasound examination [1]. Short FL is also defined using two different previously published definitions that used the biparietal diameter (BPD) to femur length (FL) ratio and the observed to expected (O/E) femur length ratio [2]. The first was described by Dicke *et al*. [3]. A femur was defined short if BPD/FL ratio was 1.5 SDs above the mean for gestational age. According O/E ratio, a short femur was found when the actual FL measurement compared to the expected femur length measurement for gestational age was ≤0.91, [4]. The regression formulae used to determine the values for expected FL measurements were derived by Nyberg *et al* on the basis of BPD: FL = −0.966 + 0.866 ×BPD; HL = −0.884 + 0.834 × BPD [5].

As previously reported, FL measurement is one of the biometric parameter measured during routine second and third trimester ultrasound evaluation to check fetal growth and to esteem fetal weight [6-8]. The detection of a fetal FL below the expected value might be a diagnostic challenge for the examiner: it may be a marker of aneuploidy or associated with other genetic abnormalities or skeletal dysplasia [1]. More recent studies have suggested as an isolated short femur, in the second trimester of pregnancy, could be an early marker of IUGR and small-for-gestational age (SGA) neonate [9-11]. In most cases, short FL may be the result of an inaccurate measurement or may be a variant of normal, especially if present as an isolated finding.

3.1 Short femur length and aneuploidies

Although chromosomal abnormalities occur at low frequency in the population, around 0.5% to 2% [12], they contribute significantly to increase perinatal morbidity and mortality [13]. Trisomy is the most frequent chromosomal abnormality, especially of chromosome 21, that is, Down syndrome (prevalence 1 in 660 live births) [14]. This syndrome was first described in 1866 by John Langdon Down who gives it the name [15]. The genetic basis of DS was discovered later in 1959 by Lejeune and colleagues by the presence of chromosome 21 in excess. [16]. Down Syndrome phenotype is complex and varies among individuals, who may present a combination of dysmorphic features and developmental delay [17]. The intellectual disability is a characteristic observed in all cases. On average, 50-70% of children with Down Syndrome have congenital heart defects, such as ventricular septal defect, atrial septal defect, tetralogy of Fallot, patent ductus arteriosus and atrioventricular septal defect [18]. The most frequent clinical features predominantly affect the head, the neck and the extremities. Changes in the extremities include short broad hands, hypoplastic mid phalanx of fifth finger, incurved fifth finger, transverse palmar crease, space between the first and second toes (sandal gap deformity), hyperflexibility of joints. In addition, fetuses with trisomy 21 have slightly shorter long bones than their normal counterparts. Actually, the mean length at birth is approximately 0.5 SDs less than normal babies. In addition birthweight and head circumference are inferior in babies with trisomy 21 when compared with normal counterparts [19].

The antepartum detection of fetal aneuploidy is one of the major goals of prenatal diagnosis and today first-trimester combined test for chromosomopaties is included in

screening programs by healthcare service. It detects about 90% of fetuses with trisomy 21 with a false-positive rate (FPR) of about 5% and is based on a calculated risk by the combination of many variables (maternal age, fetal NT and serum markers β-hCG and PAPP-A) [20]. This screening can be further improved by assessing additional ultrasound markers such as the nasal bone, Doppler flow in the ductus venosus and across the tricuspid valve [21-24]. The capabilities for screening and prenatal diagnosis of T21 have greatly improved, most recently with the implementation of noninvasive prenatal testing using cell-free fetal DNA [25]. However, for definitive diagnosis, karyotype analysis by amniocentesis or chorionic villus biopsy is needed.

Despite recent advances in first-trimester screening methods, the second-trimester sonogram continues to be an important tool in the detection of fetal trisomy 21. Multiple sonographic markers have been reported to be associated with trisomy 21 including thickened nuchal fold, hyperechoic bowel, echogenic intracardiac foci, ventriculomegaly, shortened femur or humerus length, renal pyelectasis, and absent nasal bone [26-27]. The presence or absence of these markers both isolated and in combination, can be used to adjust a woman's age-related risk so as first trimester screening risk of having a fetus with trisomy 21 [28]. The challenge is to distinguish the presence of these small alterations on the second-trimester ultrasound, between chromosomally abnormal and normal fetuses, considering that the latter may also present these markers at a rate of around 13% to 17%, which can be considered to be a high percentage of false positives [29]. The importance of this challenge is greater among pregnant women of advanced maternal age, when the relationship with Down syndrome becomes closer.

The role of the short FL in the antenatal diagnosis of Down's syndrome has been evaluated by many investigators. In 1987, Lockwood et al and Benacerraf et al were the first ones to show that short FL was associated with increased risk of trisomy 21 [3, 29]. In their studies this sign was identified in 50 to 68% of second-trimester fetuses with T21. Following studies also showed as this finding had a sensitivity of 40 to 50% and an FPR of 2.2 to 6.5% for detecting fetuses with Down syndrome [30-32]. However, the overwhelming majority of these data have been taken from high-risk patients (women >35 years old or women with abnormal serum biochemistry results). DRs for Down syndrome in sonographically screened low-risk populations have become available only recently [33,34] and controversy exists regarding the significance of sonographic markers, especially when isolated, for the detection of Down syndrome as well as the role of genetic sonography for further risk modification after first- or second-trimester combined or biochemical screening [35,36]. Vintzileos et al in 1995 summarized results of sixteen studies that used fetal femur measurement to detect T21 in second trimester. Although different methods were used to define the abnormal test, the average sensitivity was 31%. The range of sensitivities, however, was 13% to 70%. The average FPR was 5% [37]. A subsequent meta-analysis published in 2001 found a sensitivity of 16% in the prediction of Down syndrome with a FPR of 4%, when present as isolated finding [38]. A recent analysis of soft marker published in 2013 reported a likelihood ratio of 3.72 [39]. Although of limited predictive value when isolated, short FL has a good sensitivity among soft markers. Another advantage is that femoral measurements are highly reproducible [40] whereas reproducibility of other soft markers remains to be proven.

Other biometric parameters were also evaluated, but the most significant changes were observed in the length of the extremities. Most investigations of humeral length (HL) in the detection of fetal trisomy 21 have shown superior performance of this marker compared to shortened FL [41]. Anyway HL measurement is a biometric parameter not required by national guidelines in screening program [6, 7].

3.1.1 Other soft markers for aneuploidies

"Soft markers" are distinct ultrasound findings by prenatal ultrasound which may be transient, having little or no pathological significance, but are thought to be more commonly found in fetuses with karyotypic abnormalities. Given this association, these markers are of interest as potential identifiers of these conditions. The appearance of a soft marker, either singly or in combination, can lead to further targeted ultrasound evaluation, as well as adjusted counseling regarding the fetal risk of trisomy 21. Thus, on the basis of a soft marker's appearance, women may be confronted with further genetic counseling, and some will choose to undergo an invasive diagnostic procedure.

3.1.1.1 Other soft markers for aneuploidies: choroid plexus cyst

The choroid plexus is the region of the brain responsible for production of cerebrospinal fluid. A choroid plexus cyst (CPC) is a small fluid-filled structure within the choroid of the lateral ventricles of the fetal brain. Sonographically, it appears as echolucent cyst within the echogenic choroid. CPC may be single or multiple, unilateral or bilateral, and most often less than 1 cm in diameter. The prevalence is approximately 1% of fetuses in the second trimester [26]. It is a transient finding and usually disappears after 24 week of gestation. If there are no associated anomalies, CPC should be considered a normal variant. They have no known association with adverse clinical outcomes when the karyotype is normal, and they are not associated with fetal development or childhood

neurocognitive or behavior delays [42]. Studies that have investigated CPC in low-risk populations report an overall risk of an associated aneuploidy of approximately 1%. [43, 44] The most commonly associated trisomy is trisomy 18. An isolated finding without an elevated a priori risk for fetal aneuploidy does not warrant additional testing [26].

3.1.1.2 Other soft markers for aneuploidies: echogenic intracardiac focus

An echogenic intracardiac focus (EIF) is diagnosed on the standard four-chamber view as a focus of echogenicity comparable to bone, in the region of the papillary muscle in fetal heart ventricles. This finding is most commonly seen in left ventricle (88%), although 5% are only in right ventricle and about 7% are bilateral. It is commonly found in healthy fetuses (prevalence 1-3%). An EIF is not considered a structural or functional anomaly, nor is associated with heart defects or poor clinical outcome [45, 46]. It has been associated with an increased risk of T21 with a likelihood ratio of 5.83 [39]. However if isolated, it does not alter the risk of Down syndrome. In the absence of associated anomalies, therefore, the execution of the karyotype is not indicated. [27]

3.1.1.3 Other soft markers for aneuploidies: mild pyelectasis

Pyelectasis is defined as a spherical or elliptic anechoic space in the renal pelvis that measures between 5 and 10 mm. The measurement is obtained with a cross section of the renal pelvis using the maximum antero-posterior diameter. Dimension inferior to 5 mm should not be reported as pathological [26]. The fetal pyelectasis is a common ultrasound finding, identified in 0.5–5% of pregnancies. It can be unilateral or bilateral with a slightly higher incidence in male fetuses. The association with chromosomopathies was mainly found with Trisomy 21 (about 2%), especially in the presence of other risk factors (familiarity, maternal age> 35) and for a value superior to 4 mm. In the absence of risk factors, the possibility of Down syndrome in the presence

of this ultrasound sign is low and would not justify the use of invasive procedures for the study of the fetal karyotype. In most cases, pyelectasis evolves favorably, disappearing before the end of pregnancy or at most within the first 30 days of delivery [47-49].

3.1.1.4 Other soft markers for aneuploidies: single umbilical artery

The normal umbilical artery contains two umbilical arteries and one umbilical vein. Single umbilical artery (SUA) is found in cases where there are only two vases in the umbilical cord, due to lack of an artery. It is the most common anomaly of the cord, with a rate of 0.2-1.9% in singleton and 4.9% in twin pregnancy. The most common etiology of the pathology is the primary artery agenesis. Atresia or secondary atrophy of an artery normally present in early stages of development seems less likely [50]. It is common practice to visualize funicular vessels early in the first trimester with Doppler color on either side of the bladder [7]. Diagnosis became easier from the 20th week. The association between SUA and fetal pathology and aneuploidies is increased when this sign is associated to other sonographic markers of anomalies. Isolated SUA has not been found to be significantly associated with chromosomopaties. Without an elevated a priori risk for aneuploidy and no concurrent sonographic abnormalities seen on ultrasound, invasive testing is not recommended. In some studies isolated SUA has been associated to other pregnancies complication, in particular IUGR. In these patients is useful a growth scan in third trimester.

3.1.1.5 Other soft markers for aneuploidies: echogenic bowel

Echogenic fetal bowel is a sonographic finding, in which the fetal bowel appears to be brighter than normal. The intestine is defined hyperechoic when its echogenicity is equal to or greater than bone, after reducing to a minimum the gain. The echogenicity has been classified as either focal or multifocal [51]. Echogenic bowel is a subjective finding; in an effort to standardize and improve inter-observer accuracy, there are grading systems that attempt to improve classification of the echogenicity of fetal bowel. Nyberg et al proposed a classification in three degree

(a) grade 1: mild hyperecogenicity of the intestine - comparable to that of the liver - which is not attributable to pathological significance;

b) grade 2: the echogenicity of the intestine is superimposed on that of the bone;

c) Grade 3: the echogenicity of the intestine is greater than that of the bone [52].

The prevalence of this sign ranges from 0.2 to 1.8% in second trimester ultrasound. It could be a normal variant, but in about 35% is associated with underlines pathologies: IUGR, cystic fibrosis, fetal infections (cytomegalovirus, herpes virus, parvovirus, rubella, varicella and toxoplasmosis), gastrointestinal anomalies; intramniotic bleeding; aneuploidies. The presence of echogenic bowel is associated with an increased risk for fetal aneuploidy, including trisomy 13, 18, 21, and the sex chromosomes [26]. A recent meta-analysis reports a likelihood ratio for this marker of 11.44 that decreased to 1.65 if present in isolated form [39].

3.1.1.6 Other soft markers for aneuploidies: nuchal fold

Unlike NT, whose ultrasound measurement occurs during the first trimester of pregnancy, the nuchal fold (NF) indicates the thickness of the soft tissue of the neck,

assessed in the second trimester. The value of NF increases with gestational age, so it would be correct to use a different cut-off for the various gestational ages; a measurement >6 mm is considered significant between 18 and 24 weeks while a measurement of 5 mm is considered significant at 16 to 18 weeks [26]. An increase in the NF is due to multiple causes: delayed abnormal development of the lymphatic system; heart pump abnormalities; neuromuscular abnormalities; metabolic disorders; anemia from congenital or acquired causes [26]. An abnormal or thickened NF is considered to be the most sensitive and specific marker for the detection of trisomy 21 in the second trimester. The sensitivity has been reported to be 42–43%, with low false positive rates ranging between 0.1% and 1.3% [53]. The likelihood ratios (LR) is estimated to be around 23 [39]. In addition, even in the absence of aneuploidy, an increased NF remains a marker for Noonan syndrome and congenital heart defects [43, 65]. The detection of an enlarged NF during second trimester ultrasound evaluation should prompt a thorough evaluation for other fetal anomalies, a fetal echocardiogram, as well as a review of prior genetic screening. The patient should be referred for genetic counseling, undergo a thorough risk assessment, and be offered invasive testing for aneuploidies.

3.1.1.7 Other soft markers for aneuploidies: ventriculomegaly

The cerebral ventriculomegaly, most commonly alteration found in the CNS, is defined when the width of the lateral ventricle atrium is more than 10 mm. Moderate ventriculomegaly is defined as a measurement between 10 and 15 mm. Above 15 mm. we talk about hydrocephaly. The range ranges from 10 to 12 mm is considered a gray area that includes many normal and some pathological fetuses and is defined as

borderline [26]. The outcome depends on progression over time. Most of the borderline ventriculomegaly generally regress after birth and does not require special care. Ventriculomegaly is usually an isolated finding; however, it may be associated with other CNS anomalies (microcephaly, Dandy-Walker complex, spina bifida, agenesis corpus callosus), infectious disease and aneuploidies. 1.4% of trisomy 21 fetuses in the second trimester have idiopathic ventriculomegaly [54]. The LR as isolated marker is around 3.8 for the risk of karyotype abnormality [39].

3.1.1.7 Other soft markers for aneuploidies: enlarged cisterna magna

The cisterna magna contains cerebrospinal fluid and is located behind the cerebellum in the back of the brain. It communicates with the 4th ventricle through the foramen of Magendie and Luschka. The ultrasound evaluation of the cerebellum and cisterna magna is an integral part of screening between 16th and 20th week. Enlarged cisterna magna is defined by a size greater than 10 mm. An association with aneuploidies, particularly trisomy 18, has been described when this sign is present with other anomalies. In isolated form does not appear to raise the risk. Enlarged cisterna magna can be observed in association with other anatomical anomalies (arachnoid cysts, Dandy-Walker) and syndrome abnormalities (gold-facio-digital syndrome, DiGeorge syndrome, and Meckel-Gruber syndrome). If present in association with other anomalies the fetal karyotype should be proposed [26].

3.1.1.8 Other soft markers for aneuploidies: nasal bone

Nasal hypoplasia has been recognized as a feature of postnatal trisomy 21 [15]. This has led to prenatal evaluation of the nasal bone. During second trimester routine scan nasal

bone can be displayed in a mid-sagittal view of fetal face as an echogenic line distinct from the above skin. When present, the nasal bone length could be measured by placing the calipers in the out-to-out position; measurements can be compared with the normal range reported by Cicero et al. [54] and were considered to be hypoplastic when found to be less than 2.5 mm. On the basis of current evidence, the prevalence of nasal bone absence in trisomy 21 fetuses in the second trimester is 37% and 1% in euploid fetuses, resulting in positive likelihood ratios of 23.27 and 6.58 when present as isolated findings [39]. As such, it appears to be a strong marker for screening Down syndrome in second trimester. (Table b)

	Trisomy 21	**Trisomy 18**	**Trisomy 13**
Major anomalies	Cardiac defects	Cardiac defects	Cardiac defects
	Duodenal atresia	Spina bifida	Central nervous system abnormalities
	Cystic hygroma	Cerebellar dysgenesis	Facial anomalies
		Micrognathia	Cleft lip/palate
		Omphalocele	Urogenital anomalies
		Clenched hands/wrists	Echogenic kidneys
		Radial aplasia	Omphalocele
		Club feet	Polydactyly
		Cystic hygroma	Rocker-bottom feet
			Cystic hygroma
Minor Marker	Nuchal thickening	Choroid plexus cysts	EIF
	Hyperechoic bowel	Brachycephaly	IUGR
	EIF	Shortened limbs	Pyelectasis
	Shortened limbs	IUGR	Single umbilical artery
	Pyelectasis	Single umbilical artery	
	Mild ventriculomegaly		
	Clinodactyly		
	Sandal gap		
	Widened pelvic angle		
	Pericardial effusion		
	Right-left heart disproportion		

Table b . Major and minor markers associated to aneuploidies

3.2 Short femur length and skeletal dysplasia

Skeletal dysplasias represent a large, heterogeneous group of rare developmental disorders of chondro-osseous tissue that involve the formation and growth of the bone. The current "Nosology and Classification of Genetic Skeletal Disorders" includes more than 400 skeletal abnormalities divided in 42 groups [1]. Despite this considerable number, birth incidence is nearly 1/5000; stillbirths amount to 20 per 20.000 and each individual skeletal dysplasia is relative rare [2]. They represent a significant burden to many families because of potential lethality, short- and long-term medical complications. Although family history plays an important role in assessing risk of skeletal dysplasias, many of these disorders result from new dominant mutations and from autosomal recessive diseases, in the absence of any known parental risk factors [3]. Prenatal ultrasound (US) has an accuracy of 65–68% for diagnosis of a particular skeletal dysplasia [4, 5]. Many of the prenatal onset skeletal dysplasias are associated with lethality because of pulmonary insufficiency or concomitant visceral abnormalities [6]. In these cases prenatal accuracy could approach to 100% in fetal medicine specialized center thanks to knowledge of the appropriate diagnostic criteria. Currently, the gold standard for diagnosis of a skeletal dysplasia includes a combination of antenatal ultrasound (US), postnatal radiologic features, pathology and cytogenetic evaluation. Suspicion of a fetal skeletal dysplasia is usually prompted by identification of long-bone shortening on sonography [7]. The fetal skeleton is easily visualized by two-dimensional ultrasound early in gestation. Of note, the long bones, vertebrae and calvarium begin ossifying by 12 weeks. Measurement of fetal femur is considered part of any basic ultrasound evaluation. Any fetus showing femora or humeri length measurements less than 5th centile or 2 SD from the mean in the second trimester

should be evaluated in a center that has expertise in evaluating the entire fetal skeleton and has the ability to provide genetic counseling. The following fetal ultrasound measurements should be visualized and plotted against normative values: fetal cranium (biparietal diameter and head circumference), facial profile, mandible, clavicle, scapula, chest circumference, vertebral bodies, all fetal long bones, and the hands and feet.

Comparison of the relative length of all the long bones and against normative values will determine whether there is primarily rhizomelia, mesomelia, or that both segments are involved. Although there is severe shortening of all limbs in the majority of skeletal dysplasias, the foot length is relatively normal. In addition to measurement of the length, the long bones should be evaluated regarding changes in shape, mineralization, bowing, angulation, and metaphyseal flare. If isolated short femurs are found bilaterally, one should initially consider more common etiologic factors, including inaccurate gestational dating, ethnic variation, soft markers for aneuploidies, or fetal growth restriction. Diagnosis of skeletal Dysplasia is more probable in the subsequent condition:

- marked and early shortening of the long bones (< -3DS)
- disproportionate between long bone measurement and fetal abdominal and calvarial growth, especially if the head circumference is greater than the 75th centile
- not proportioned femur to foot ratio (which approaches 1.0 throughout gestation)
- pattern of growth in lethal dysplasias continues to decelerate throughout fetal life

3.3 Short femur length and intrauterine growth restriction

Recent studies have shown an association between short isolated femur, that is a short FL not associated with aneuploidy or skeletal dysplasia, and intrauterine growth delay [1-4].

IUGR complicates about 5-10% of pregnancies and is associated with poor perinatal outcome [5]. It refers to a condition in which a fetus is unable to achieve its genetically determined potential size, because of a pathologic process. The most widely used definition of IUGR is an estimated fetal weight less than the 10th percentile for gestational age [6]. This definition include also SGA fetuses that are not pathologically small but at the lower end of normal range [6, 7].

Two different types of intrauterine growth delay could be identified:

- *asymmetrical* (or late flattening), characterized by a rapid decline in growth during the third trimester. It is usually manifested after the 26th week, it gets worse proceeding in gestation and is caused by placental insufficiency secondary to maternal diseases such as hypertension, diabetes, thrombophilia and thalassemia. In the case of asymmetric IUGR, an increase in the resistance index and a decrease in flow velocity in splanchnical and muscular districts are observed in favor of flux redistribution for encephalic, cardiac and adrenal districts.

- *Symmetric* (or low profile), defined by a growth rate constantly below the normal range. It is associated with congenital diseases or infections contracted early in pregnancy. It can also be of family or constitutional origin. IUGR is defined as "severe" when its biometric determination is <3 percentile and "mild" when it is between 3 ≤ and ≤ 10 percentile [8].

In placental defects, resulting in placental insufficiency, highly oxygenated fetal blood from abnormal placenta is mainly directed towards vital organs such as the heart and nervous system at the expense of the extremities [4]; uteroplacental insufficiency is firstly indicated by a reduced fetal urine production and oligohydramnios due to fetal blood flow redistribution followed by an asymmetrical growth restriction. In case of asymmetrical IUGR the abdominal circumference is usually the first parameter to decrease due to diminished glycogen storage in the fetal liver [9]. However, a few recent studies indicate that a short femur is an early marker of IUGR caused by an impaired placental function [1-4]. Dysfunctional placenta seems to produce altered levels of growth factors involved in normal fetal skeletal development [9]. Todros et al. argued that the short femur was due to an altered secretion of the type 2 fibroblast growth factor by the abnormal placenta [2]. Cases of isolated short FL were associated with significantly lower levels of PAPPA, but similar β-hCG, inhibin-A, and alpha-fetoprotein (AFP) level when compared to fetuses with normal FL [10]. According to some studies in the literature, the short isolated femur allows to identify 66% fetuses with IUGR and SGA with a specificity of 67% [11]. The short isolated femur is therefore an early marker of placental dysfunction. It is associated with an increased risk of subsequent development of IUGR, but also an increase in the risk of preterm delivery and adverse pregnancy outcome [3,4]. Maternal evaluation regarding blood pressure monitoring, follow-up for development of preeclampsia, ultrasonographic evaluation of the placenta, and Doppler analysis should be offered in this situation.

AIM OF THE STUDY

Fetal short femur is defined by a FL below the 5th percentile or -2 DS for the gestational age. The finding of a short femur often represents a diagnostic dilemma for the clinical geneticist and the expert in fetal medicine for the various differential diagnosis. Such finding may be associated with congenital anomalies such as skeletal dysplasia, aneuploidies or genetic pathologies. In the isolated form, it may be an early sign of placental insufficiency and growth delay, or a normal variant in constitutionally small fetuses [1, 2].

Clinical experience leads us to find this ultrasound sign at all stages of pregnancy, often with difficulties in counseling the couple about the prognosis, especially when present in an isolated form. Despite the arising scientific literature on the subject, there are currently no studies comparing perinatal outcome of the short fetal femoral diagnosed during different gestational ages.

The aim of this study was:

- ✓ to assess the prevalence of short FL in a cohort of pregnant women referred to a specialized Centre for Prenatal Diagnosis for screening or second level ultrasound examination.
- ✓ To examine natural history and postnatal outcome of pregnancies complicated by a short femur length;
- ✓ to compare outcomes in pregnancies with an early diagnosis of short FL (< 24 weeks of gestation) with pregnancies where this sign arises later in gestation (> 25 weeks of gestation).
- ✓ to analyse outcome differences in isolated and non-isolated form.

A secondary aim of our research was a proposal of a diagnostic algorithm as a tool to

guide clinicians in the management and counselling of pregnancy with isolated and not isolated short femur length. For this purpose a revision of current literature data on the argument was carried out. We reviewed the literature and used meta-analytic technique to estimate accuracy of this marker in the prediction of Down Syndrome, IUGR and skeletal dysplasia. Correlation with poor perinatal outcome was also evaluated.

METHODS

This was a longitudinal prospective cohort study of all cases of short FL, diagnosed from January 2012 until July 2017, in Prenatal Diagnosis Centre, Policlinico Umberto I Hospital, 'Sapienza' University of Rome. This is a referral centre for high risk pregnancies where fetuses with a diagnosis or a suspicion of fetal anomaly are referred for further detailed assessment. All patients undergo a thorough biometric and anatomic evaluation by certified obstetric sonographers, and all images are evaluated by maternal–fetal medicine specialists. For all gestations seen in this center, pregnancy is dated by Last Menstrual Period. In cases in which the gestational age is discordant with the first-trimester CRL by more than seven days, the gestational age is based on CRL measurement. For patients who do not have a first-trimester ultrasound performed, menstrual dating is used as a reference if it is consistent with the acquired biometry. If a biometric discrepancy emerges, a combination of single parameters, BPD, Head Circumference (HC), abdominal circumference (AC), and FL, is used to determine gestational age, rather than a single parameter [3]. Every biometric evaluation is realised according to the standards set within the national and international guidelines [3, 4].

All singleton pregnancies with a diagnosis of fetal femur < 5 centile were enrolled in the study. For every patient a detailed anamnesis was performed. Pregnancy documentation on previous ultrasound scans and prenatal diagnosis exams were consulted and

information of note reported in medical register. Subsequently an ultrasound examination was performed to confirm the FL measurement and to examine for further fetal structural abnormalities. All ultrasound examinations were performed with a Voluson 730 Expert GE and Samsung Elite WS80A ultrasound machine. Gestational age-specific biometry values were determined by standards derived by Hadlock [5] and recorded in View Point software. If a FL below < 5th percentile was confirmed, a complete evaluation of fetal bone biometry (including measurements of humerus, mesomelic bones, feet and thorax circumference) was performed. A complete Doppler evaluation was offered to these patients which includes:

- registration of pulsatility index in umbilical artery and in middle cerebral artery;
- registration of pulsatility index and resistance index in both uterine arteries.

Serial Doppler studies and growth evaluation were performed every two week.

A genetic counseling was proposed to all couple both in presence of additional fetal anomalies than in isolated cases of short FL. Karyotype analysis was offered whenever a malformation was detected. The possibility of an invasive procedure for fetal sampling and genetic analysis was discussed in case with isolated short FL. A search in our database was performed to identify all cases of short FL diagnosed in our institution during the study period. For selected cases we collected general information (age, race, family history, obstetrical history, anthropometric data of the couple) and data on obstetrics outcome (pregnancy complications, mode of birth, age at delivery, Birth Weight (BW) and Length, one and five-min Apgar score, Neonatal Intensive Care Unit (NICU) admissions, need for further investigation (including amniocentesis, fetal magnetic resonance, fetal echocardiography and genetic counseling). Information were extracted from the department's database or obtained from questionnaire, telephonic

contact with the patients, or referring provider if necessary. Patients with incomplete information were excluded from the study. Twin pregnancies were not considered in this data analysis.

All enrolled patients were divided into two groups: patients with diagnosis of FL < 5th percentile at 14-24 weeks (group A) and at 25-40 weeks (group B). The differences in pregnancy complications and outcomes between the two groups were analysed.

For the study the subsequent definitions were used:

- *Isolated short femur*: bilateral and symmetrical FL< 5 percentile in absence of associated fetal abnormalities.

- *Not isolated short femur*: FL < 5 percentile for gestational age in association to an increased NT above 3 mm at 11 to 13 + 6 weeks scan, or in presence of fetal abnormalities.

- *IUGR*: fetal weight < 5th percentile in association to abnormalities in fetal Doppler parameters.

- *SGA:* fetal weight < 10th percentile in absence to abnormalities in fetal Doppler parameters.

- *LBW*: birth weight < 2500 g.

For the secondary aim of our study we extract relevant citation from PubMed, EMBASE, and Medline to identify English language published articles that describe the correlation between:

a) fetal femur and down syndrome
b) short FL and Skeletal Dysplasia;
c) short fetal femur and IUGR/SGA.

Preliminary keywords and MeSh terms were combined to generate lists of studies: “Fetal femur and Down Syndrome”, “Fetal femur and Skeletal Dysplasia”, “Short fetal femur and IUGR/SGA”. No restriction about date of publication was posed for our research.

a) Fetal femur and Down syndrome: Inclusion criteria for study selection were: singleton pregnancy; minimum and maximum gestational age at examination between 14 and 37 weeks. Studies on second-trimester sonographic markers were eligible if first, they included and described FL in both euploid and trisomy 21 fetuses, second, the fetal karyotype was unknown at the time of sonographic examination (to avoid overt diagnosis bias) and third, chromosomal status of the fetuses was confirmed by either karyotype (the gold standard) or postnatal clinical examination. Data in the euploid group were stratified for pregnancies at high or low risk. Women at high risk were defined as women who underwent amniocentesis for advanced maternal age, positive first or second-trimester maternal serum screen for a chromosomal anomaly, the detection of a major congenital anomaly, sonographic markers of fetal aneuploidy or a positive family history of a previous pregnancy affected by a chromosomal abnormality.
b) Short femur length and Skeletal Dysplasia: Inclusion criteria for study selection were: singleton pregnancy, minimum and maximum gestational age at examination between 14 and 37 weeks. Studies were eligible if first, they included and described FL in Skeletal Dysplasia, second, the fetal anomaly was unknown at the time of sonographic examination (so as to avoid overt diagnosis bias) and third, the diagnosis was confirmed by either genetic test (the gold standard) or postnatal clinical examination.

c) short femur length and IUGR/SGA: inclusion criteria for study selection were: singleton pregnancy, minimum and maximum gestational age at examination between 18 and 24 weeks. Studies were eligible if first, they included and described association between short FL and IUGR/SGA. Studies reporting pregnancy and perinatal outcomes in term of hypertensive disorder, intrauterine death, preterm birth, Apgar Score at 5 minutes inferior 7, low birth weight, NICU admission.

Prospective and retrospective cohort studies were considered eligible for inclusion if the above criteria were met. Personal communications, letters, case-reports and non-English language publications were also excluded. Two authors independently reviewed articles and abstracted data. Discordance was resolved with consensus.

Exclusion criteria were: omitting at least one inclusion criterion, and data reported in graph or percentage form rather than proportional rates. Quality and integrity of this review were validated with PRISMA: preferred reporting items for systematic reviews and meta-analyses [6]

Statistical analysis

Variables measured in interval scales were described as the mean plus or minus the standard deviation (SD). Data points, collected for this study, were analyzed using the Fisher test and Chi-square test to compare proportions, as appropriate Statistical significance was set at p values lower than 0.05. All P values presented were two sided, and associations were considered significant if the P value was \ 0.05. SPSS statistical software (IBM, Ar- monk, NY) was used.

A meta-analysis was performed to provide performance of short femur length, as sonographic marker to identify Down syndrome skeletal dysplasia and IUGR/SGA fetuses. We assessed the overall diagnostic performance by weighted independent estimation of detection rate (sensitivity), false-positive rate (1−specificity) and odds Ratio. We used both fixed and random effects models to estimate weighted detection rate, false-positive rate and odds ratio across studies. The fixed-effects model weighs each study by the inverse of its variance. Random effects incorporate both within-study and between-study variation. Random effects tend to provide wider CIs and are generally preferable, especially in the presence of between-study heterogeneity. Heterogeneity between studies was analyzed using both Higgins' $I2$ and Q-test and was considered to be high if $I2$ was over 0.5051. To explore the potential effect of different study populations on heterogeneity we performed such analysis for the whole dataset and in the subgroups of studies classified as high risk and screening for Down syndrome. The statistical software package SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and Meta-Analyst (Tufts Medical Center, Boston, MA, USA) were used for data analysis.

RESULTS

During the study period 5140 women were referred for ultrasound scan in our centre. A short FL was identified in 260 cases (5%). Of these 67% (200/260) were single and 23% were (60/260) multiple pregnancies. Multiple pregnancies did not meet the inclusion criteria for the study. 53 singleton were also excluded due to insufficient data on pregnancy and neonatal outcome. 147 cases remained for the analysis. Mean maternal age was 33 years (range 17-47) and 36 % were over 35 years old (53/147).

More than half of patient were nulliparous (87/147, 59.1%), 30,6% secondiparous, and only 15/147 patients (10,2%) have already more than two pregnancies. Women were more likely to be Caucasian (White, 94%; Black, 1.4%; Asian 4.6%). Patients' demographic characteristics were similar to normal population in terms of maternal weight, height and body mass index. Thirty patients were smoker (20%). Gestational hypertensive disease recurred in about 13% of patients, a prevalence similar to general population [7]. Mean gestational age at the diagnosis of a short FL was 26 (range 14-37). Demographic characteristics of the study population were reassumed in table 1.

In 61 (41,49%) cases short femur was associated to other fetal anomalies: 21/61 (34%) had chromosomal abnormalities, 16/61 (26,2%) presented a skeletal dysplasia, in 24/61 (39,3%) fetuses other structural malformations were diagnosed. The skeletal dysplasia consisted of 4 cases of achondroplasia, 2 cases of osteochondrodysplasia, 2 cases of PFFD (proximal focal femur dysplasia), 2 cases of osteogenesis imperfecta. 6 fetuses had an unclassified skeletal dysplasia. Prenatal karyotyping revealed 10 cases with trisomy 21, 6 with trisomy 18/13 and 5 with other aneuploidies. In 86/147 fetuses (58,5%) short fetal FL was classified as isolated. Firstly patients were divided in two groups according to gestational age at the diagnosis: Group A include patients with an early diagnosis of short FL (14-24 weeks of gestation) and Group B patients in which this sign appeared later in gestation (25-37 weeks of gestation). Group A consisted in 66 cases (44,9%) and group B in 81 cases (55,1%). Fetal and perinatal outcomes were compared in the two groups. Abnormal fetal karyotype (27,3% vs 3.7% p: 0.02) and skeletal dysplasia (19,7% vs 3.7% p: 0.002) were more frequent in group A. Cases of multiple abnormalities was diagnosed in 9 cases in group A and in 6 cases in group B with a difference not statistically significant (13.6% vs 7.4% $p < 0.193$). Diagnosis of

isolated short femur was more common in group B (79% vs 33,4%, p: 0.000). In group B diagnosis of IUGR was made in 44.4% vs 19.7% of group A (p:0.002). The SGA prevalence had a difference statistically significant between the two groups (7.6% vs 24.7% p:0.007) (Table 2). In group A we found a poor outcome of pregnancies with an high rate of abortion and fetal demise compared with group B but this difference was not statistically significance (12,1% vs 1,2% p 0.011). The percentage of live birth was significant lower than group B (34.8% vs 97,6%). 35 patients (53.1%) opted for interruption of pregnancy for therapeutic reasons in this group due to fetal anomalies (Table 3).

In a second step analysis new two groups were created in our population to compare outcome on the basis of presence of short femur as isolated or not isolate finding (Group 1: Isolated - Group 2 not isolated). Abnormal fetal karyotype and (24,6% vs 7,0% p: 0.004), skeletal dysplasia (24,6% vs 1.2% p: 0.004) were more frequent in non isolated group. Diagnosis of IUGR and SGA was more common in isolated group (47,7% vs 13,1%, p: 0.000, 25,6% vs 4,9% p 0.001) (table 4). The percentage of live birth was significant lower in not isolated group (45.9% vs 86% p 0.00). 29 (48%) patients opted for TOP in this group due to fetal anomalies. Not statistically difference were noted in terms of fetal demise and abortion (table 5).

In our population we registered 102 live born fetuses (69,38%), 36 (24,48%) TOP and 9 fetal demise (6,12%). The percentage of preterm birth was higher than general population (40,19%). The rate of caesarean section was higher (70/102- 68,62%) than vaginal delivery (32/102- 31,37%). Perinatal outcome in our population is reassumed in table 6. No difference in terms of perinatal outcome were found both in group A and B than in isolated and not Isolated cases. (Table 6 a,b).

A higher incidence of neonatal complication, postnatal surgery and neonatal death were notice in not isolated group compared to isolated (57,69% vs17.45% p 0.019; 27,92% vs 4,2% p:0.003). (Table 7 a,b)

Table 1. Demographic characteristics of the study population

Variable	***Median (IQR) or* n *(%)***
Maternal age	33 (17-43)
Maternal age Over 35	53 (36%)
Race: • *White* • *Black* • *East Asian* • *South Asian*	 139 (94,0) 2 (1,4) 1 (0,6) 4 (4,0)
Parity • *nulliparous* • *secondiparous* • *multiparous*	 87 (59,18) 45 (30,61) 15 (10,21)
Maternal pre-pregnancy weight	61,50 +/-13,445 DS (42-115)
Maternal height	163,19 +/-6,299 DS (147-177)
Paternal height	174,917 +/-16,617 (70-198)
Paternal weight	81.13 +/-14,704 (55-175)
Maternal tabacco use	30
Pregnancy hypertension disease	19
GA diagnosis of femur length • *14-24* • *25-37*	 66 81
Normal Karyotype *Abnormal Karyotype* *Skeletal Dysplasia* *Other Fetal anomalies* *IUGR* *SGA*	126 (85,7%) 21(14,3%) 16 (16,3%) 24 (10,2%) 49 (33,3%) 25 (17,0%)

Continuous data are presented as median
Abbreviation : GA (gestational age), IUGR (Intrauterine Growth Restriction), SGA (Small for Gestational Age)

Table 2. Outcome of pregnancy - Group A and B

Outcome of pregnancy	**FL<5th diagnosed at 14-24 GA (n=66) n (%)**	**FL<5th diagnosed at 25-37 GA (n=81) n (%)**	**p value[*a]**
Isolated short femur	22/66 (33,4%)	64/81(79,0%)	0.000
Non isolated short femur	40/66(60,6%)	12/81(14,8%)	0.000
IUGR	13/66 (19,7%)	36/81(44,4%)	0.002
SGA	5/66 (7,6%)	20/81 (24,7%)	0.007
Multiple abnormalities	9/66(13,6%)	6/81(7,4%)	0,276
Skeletal abnormalities	13/66(19,7%)	3/81(3,7%) (%)	0,003
Abnormal fetal karyotype	18/66(27,3%)	3/81(3,7%)	0.000
Unexplained Short femur	2/66(3,0%)	10/81(12,3%)	0,041
Other	6/66 (9.1%)	3/81 (3,7%)	0,3

Abbreviation : GA (gestational age), IUGR (Intrauterine Growth Restriction), SGA (Small for Gestational Age)

Table 3. Final Outcome of pregnancy - Group A and B

Final Outcome of pregnancy	**FL<5th diagnosed at 14-24GA (n=66) n (%)**	**FL<5th diagnosed at 25-37GA (n=81) n (%)**	**p value[*a]**
Abortion/intrauterine demise	8 (12,1%)	1 (1,2%)	0.011
TOP	35 (53,1%)	1 (1,2%)	0.000
Born Alive	23 (34,8%)	79 (97,6%)	0.000

Abbreviation : GA (gestational age), TOP (therapeutic termination of pregnancy).

Table 4. Outcome of pregnancy. Group 1 and 2

Outcome of pregnancy	**FL<5th isolated (n=86) n (%)**	**FL<5th not isolated (n=61) n (%)**	**p value[*a]**
IUGR	41/86 (47,7%)	8/61(13,1%)	0.000
SGA	22/86(25,6%)	3/61(4,9%)	0.001
Skeletal abnormalities	1/86(1,2%)	15/61(24,6%)	0.000
Aneuploidies	6/86(7,0%)	15/61(24,6)	0.004
Fetal malformations	1/86(1,2%)	15/61(24,6)	0.004
Unexplained Short femur	10/86(11,6%)	2/61(3,3%)	0.123
Other	6/86(7,0%)	3/61(4,9%)	0,736

Abbreviation : GA (gestational age), IUGR (Intrauterine Growth Restriction), SGA (Small for Gestational Age)

Table 5. Final Outcome of pregnancy. Group 1 and 2

Final Outcome of pregnancy	**FL<5th isolated (n=86) n (%)**	**FL<5th not isolated (n=61) n (%)**	**p value[*a]**
Abortion/intrauterine demise	6 (6,9%)	3(4,9%)	0.737
TOP	7(8,1%)	29(4,7%)	0.000
Born Alive	74(86%)	28(45,9%)	0.000

Abbreviation : GA (gestational age), TOP (therapeutic termination of pregnancy).

Table 6. Perinatal Outcomes

Variable	***Median (IQR) or* n *(%)***
Birth weight in grams	2185 (450 -3700)
Birth height in cm	44,6 (29-51)
Gestational age at delivery	36 (23-41)
Preterm birth	41/102 (40,19)
Preterm birth ≤28	5 (4.90)
Preterm birth 29-34	18 (17,64)
Preterm birth 35-37	27 (26,4)
Abortion or fetal demise	9/147 (6,12)
TOP	36/147 (24,48)
Mode of delivery, n (%)	
Vaginal delivery	32/102 (31,37)
Scheduled cesarean section	28/102 (27,45)
Emergency cesarean section	42/102 (41,17)

Table 6a. Perinatal Outcomes Group A and B

Perinatal Outcomes	***FL<5th diagnosed at 14-24w***	***FL<5th diagnosed at 25-37w***	***P***
Birth weight in grams	2119	2204	0,46
Birth height in cm	45	44	0,36
Gestational age at delivery	35	36	0,33
Preterm birth			
Preterm birth ≤28	2(8,7%)	3 (3.8%)	0.818
Preterm birth 29-34	4 (17,4%)	14 (17.7%)	
Preterm birth 35-37	6 (26,1%)	21 (26.6%)	
Mode of delivery, n (%)			
Vaginal delivery	10/23(43,47%)	22/79 (27,84%)	0.362
Scheduled cesarean section	5/23(21,17%)	23/79 (29,11%)	
Emergency cesarean section	8/23 (34,78%)	34/79 (43.03%)	

Table 6b. Perinatal Outcomes Group 1 and 2

Perinatal Outcomes	***FL<5th isolated***	***FL<5th not isolated***	***P***
Birth weight in grams	2202	2139	0.720
Birth height in cm	44,41	45,46	0.365
Gestational age at delivery	36.55	35.54	0.181
Preterm birth			
Preterm birth ≤28	2(2.7%)	3 (10.7%)	0.285
Preterm birth 29-34	13 (17,6%)	5 (17.9%)	
Preterm birth 35-37	22 (29.7%)	5 (17,9%)	
Mode of delivery, n (%)			
Vaginal delivery	26	6	0,1394
Scheduled cesarean section	17	11	
Emergency cesarean section	33	9	

Table 7a. Neonatal complication Group 1 and 2

Variable	***FL<5th isolated***	***FL<5th not isolated***	***P***
5 minute Apgar Score < 7	0/71 (0%)	2/26 (7,7%)	0.069
NICU Admission	31/71(43,7%)	16/24 (66.7%)	0.062
Other complication	25/71 (35%)	15/26 (57,69%)	0.019
Surgery	3/71 (4,2%)	7/26 (26,92)	0.003

Table 7b. Neonatal complication Group 1 and 2

Neonatal complication	***FL<5th diagnosed at 14-24 GA***	***FL<5th diagnosed at 25-37 GA***	***P***
5 minute Apgar Score < 7	1/20 (5%)	1/75 (13%)	0.387
NICU Admission	8/20 (40%)	39/75 (52%)	0,452
Other complication	8/20 (40%)	32/75 (43%)	1,0
Surgery	3/20 (15%)	7/75(9.6%)	0,444

	FL<5th diagnosed at 14-24 GA	***FL<5th diagnosed at 25-37 GA***	***P***
Neonatal Death	2/22 (9%)	7/80 (8,75%)	1
	FL<5th isolated	***FL<5th not isolated***	
Neonatal Death	2/76 (2,6%)	7/26 (26,92%)	0.003

Meta-analysis

Fetal femur and Down Syndrome

Our preliminary literature search identified 110 publications. We selected 31 potentially eligible studies [8-38]. Of these 3 studies were excluded: 1 for unclear data reported, another because the study was conducted on necroscopic samples, the latter because the population include only twin pregnancies. We found nine additional studies that elude first step research but met the review inclusion criteria [39-47]. In total we included 37 qualifying studies in our analysis (Figure A).

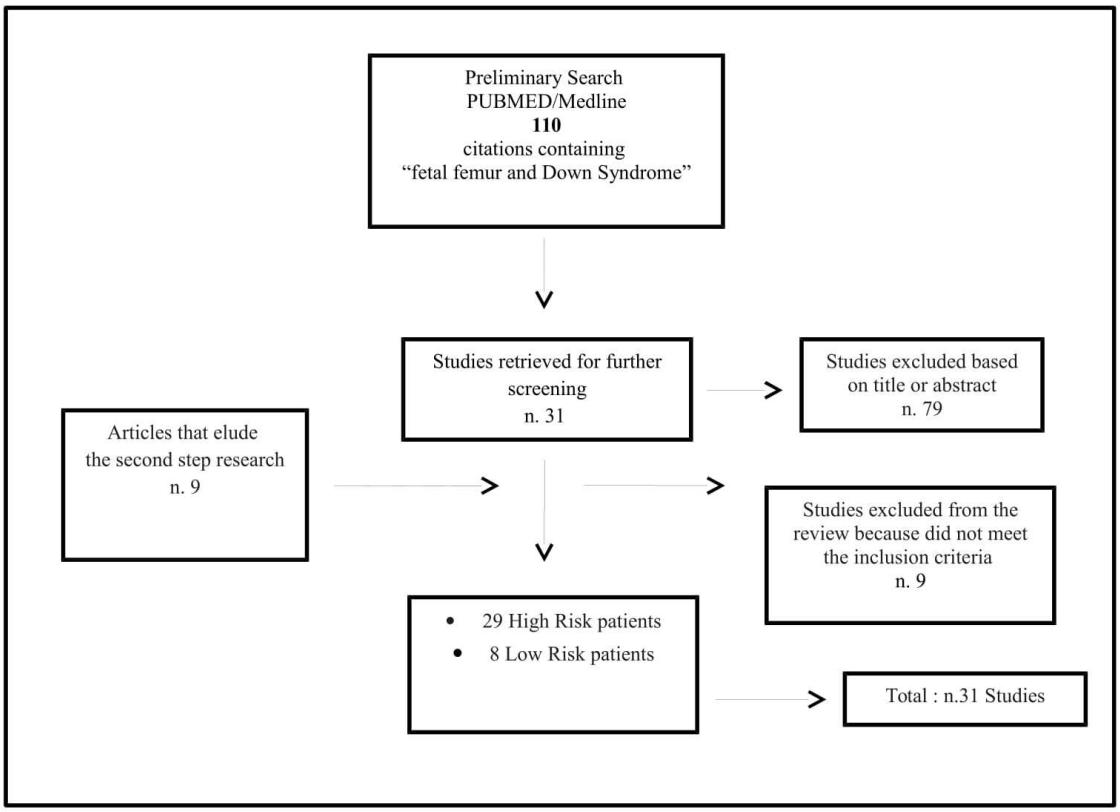


Figure A. Flow chart depicts the search strategy we used to select study for meta-analysis on fetal femur and Down Syndrome

In these studies 1326 cases of Down Syndrome (study group) and 188935 euploid controls group were described. The overall prevalence of Down syndrome was 0.7% (compared with 0.1% in general population) [48].

We analyzed 17 case-control studies, 3 cohort study, 1 multicenter study, 10 prospective and 6 retrospective. Short femur was more frequent in prospective studies 4050/32742 (12,37%) compared to case-control studies 841/9318 (9,03%) and retrospective studies 2947/151037 (1,95%).

Table 8 shows characteristics of the included articles.

Overall Down syndrome fetuses were pooled , of which 375 cases presents short fetal femur. In control group (euploid fetus) this sign was present in 5809 fetuses.

The detection rate (DR) and the FPR, for the ability of short FL to detect cases of Down syndrome, was reported for each study.

Meta-analysis showed a higher incidence of short FL in the study group (375/1326 28,2%) compared with the control group (5809/188935, 3.07%) with an OR 5.12 (95% CI, 4.47-5.87). The results showed moderate heterogeneity (value 74%). (Figure B).

Data were subsequently stratified in two groups: studies including analysis in high-risk pregnancy (29/37 - 78%) and low-risk pregnancy (8/37 - 22%). OR for high- risk pregnancy was 6.01 (5.13 - 7.04) versus 4.09 (95% CI, 3.01 - 5.55) of low risk population with a difference statistically significant between the two groups (Chi-square: 16.7766; p 0.000042, result significant at $p< 0.05$). (Figure C, D)

Table 8. Description of included studies

Study	*Year*	*Design*	*Population*	*n/N(DR)*	*n/N(FPR)*
Lockwood	1987	case-control	High-risk	32/55	35/544
LaFollette	1989	retrospective study	Low-risk	4/30	27/229
Dicke	1989	Prospective	Low-risk/high-risk	5/33	18/177
Hill	1989	case-control	Low-risk	4/22	6/286
Cuckle	1989	case-control	low-risk	20/83	84/1360
Benacerraf	1989	case-control	High-risk	7/20	28/709
Brumfield	1989	case-control	High-risk	6/15	1/45
Marquette	1990	cohort study	High-risk	3/31	14/155
Nyberg	1990	case-control	High-risk	7/49	35/572
Shah	1990	case-control	Low-risk/high-risk	3/17	1/17
Ginsberg	1990	case-control	High-risk	5/11	14/212
Grist	1990	Prospective	High-risk	3/6	25/428
Rodis	1991	retrospective study	High-risk	2/11	95/1890
Benacerraf	1991	case-control	High-risk	10/24	40/400
benacerraf	1992	case-control	High-risk	23/32	63/588
Lockwood	1993	cohort study	High-risk	6/42	163/4949
Nyberg	1993	case-control	High-risk	11/45	44/942
Campbell	1994	Prospective	High-risk	3/6	20/264
Benacerraf	1994	case-control	High-risk	20/45	4/106
Biagiotti	1994	case-control	High-risk	13/27	60/500
Nyberg	1995	Prospective	High-risk	5/18	14/232
Johnson	1995	case-control	High-risk	15/36	127/794
Grandjean	1995	prospective	High-risk	15/34	495/2763
Vintzileos	1996	Prospective	High-risk	5/22	50/493
Bromley	1997	case-control	High-risk	25/53	14/177
Nyberg	1998	case-control	High-risk	30/142	43/930
Verdin	1998	case-control	High-risk	6/11	5/449
Sohl	1999	Prospective	High-risk	9/55	42/2639
Wax	2000	prospective	High-risk	3/7	2/772
Viora	2001	prospective	High-risk	10/33	213/2069
Bahado-Singh	2002	Prospective	High-risk	30/108	503/5619
Weisz	2007	retrospective study	low-risk	1/12	111/2320
Vergani	2008	cohort study	High-risk	4/24	145/1110
Bottalico	2009	retrospective study	High-risk	2/12	7/628
Aagaard-Tillery	2009	multicentric-study	low-risk	16/56	514/7761
Rumi Kataguri	2014	retrospective study	High-risk	1/31	2/858
Mathisien	2014	retrospective study	Low -risk	11/68	2695/144948

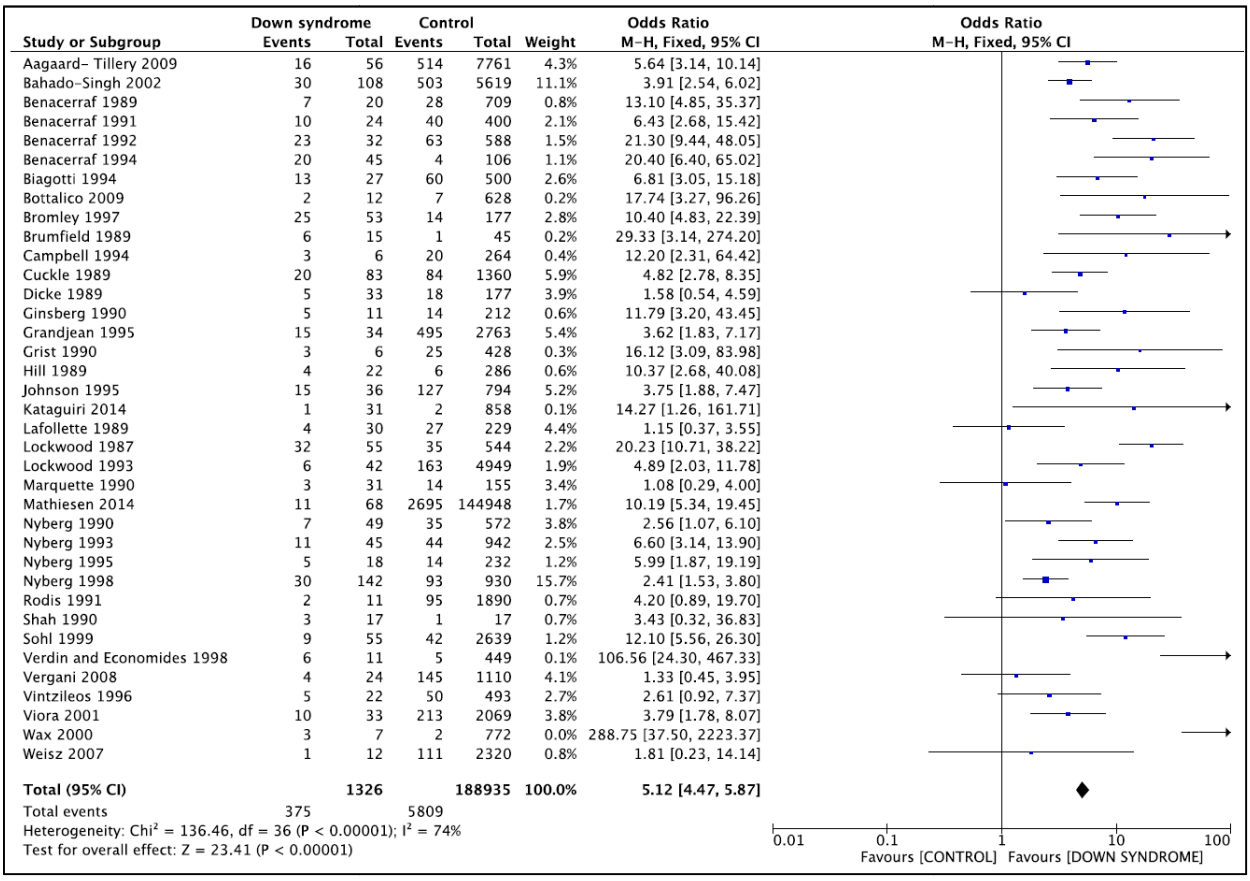


Figure B. Meta-analysis : performance of short femur lenght in screening for trisomy 21.
All studies Cases (Down Syndrome Fetuses) - Controls (Euploid fetuses).

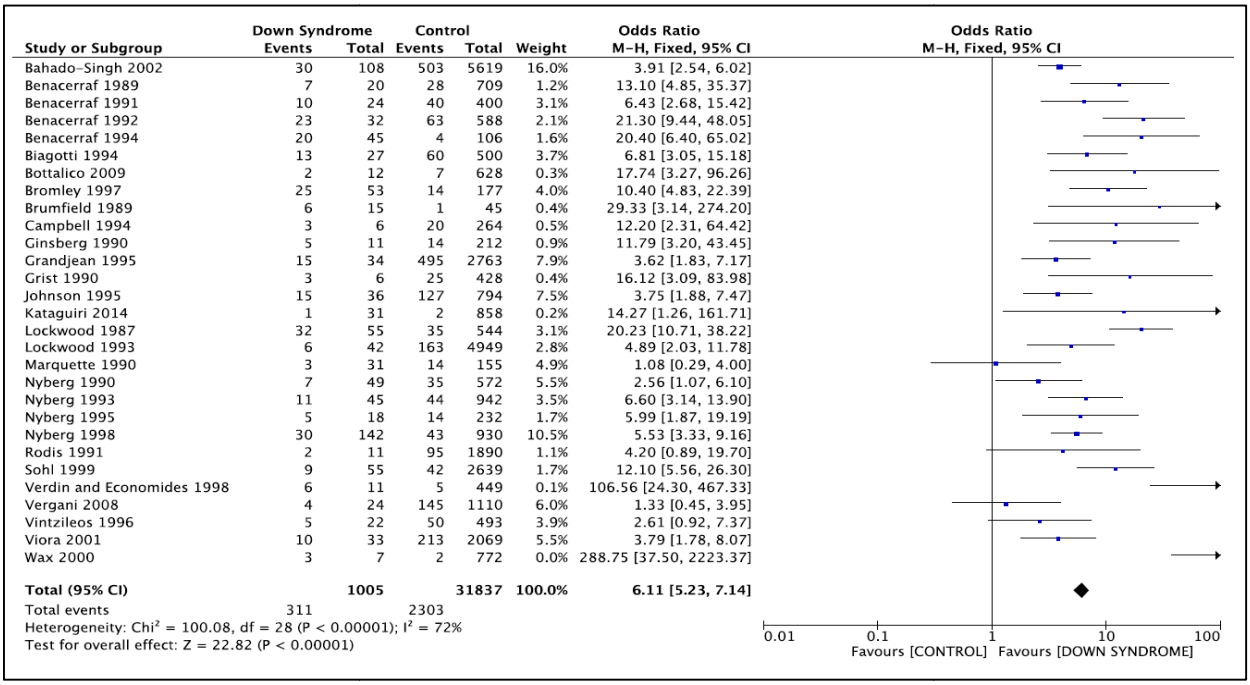


Figure C. Meta-analysis Performance of short femur length in screening for trisomy 21 in High-Risk population. Cases (Down Syndrome Fetuses) - Controls (Euploid fetuses).

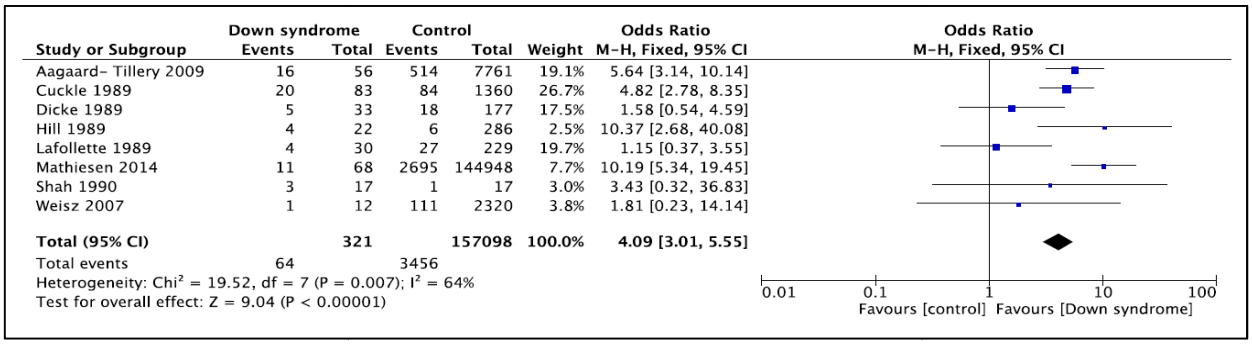


Figure D. Meta-analysis : Performance of short femur lenght in screening for trisomy 21 in Low-Risk Population. Cases (Down Syndrome Fetuses) - Controls (Euploid fetuses).

Short femur length and Skeletal Dysplasia

Our preliminary literature search identified 41 publications. 35 studies were excluded because they didn't meet inclusion criteria. In particular many studies were short reports or review. We selected 5 potentially eligible studies [49-53]. We found one additional study that elude first step research [54] (Figure E). A meta-analysis could not be conducted because of the heterogeneity of the studies. Studies selected did not show uniformity and data was not similar enough to combine for statistic meta-analytic analysis.

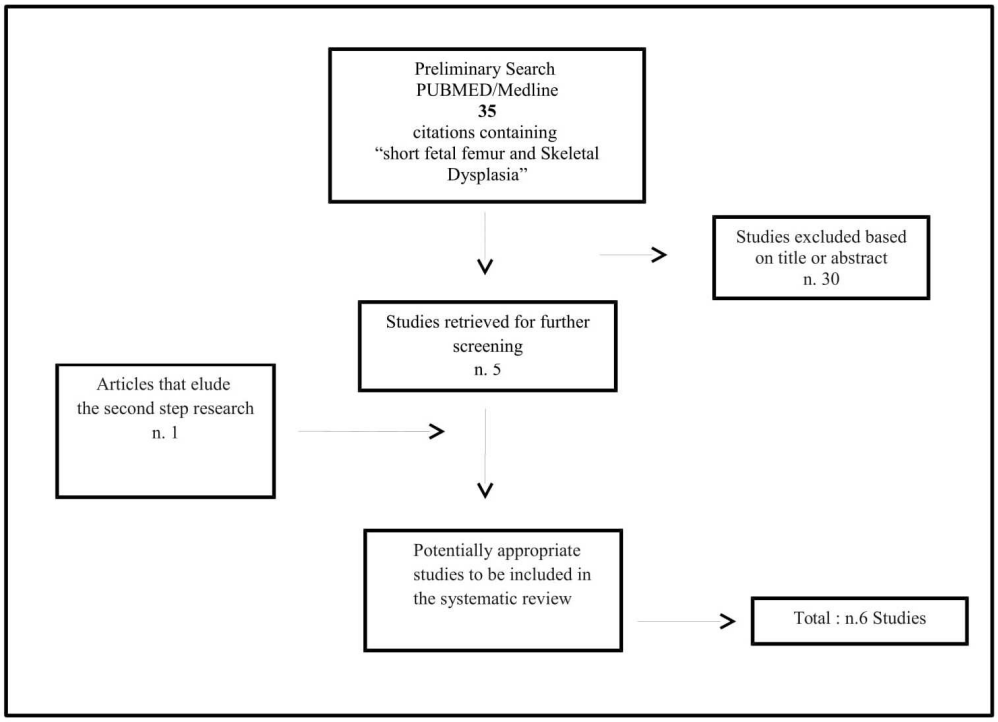


Figure E. Flow chart depicts the search strategy we used to select study for review on short fetal femur and Skeletal Dysplasia

In particular:

- Morales-Roselló et [49] al did not find any case of skeletal dysplasia in their population of isolated short femur in mid-trimester ultrasound;
- Papageorghiou et al [50] found 35% (16/46) of Skeletal dysplasia in their population of non isolated short femur (Asphyxiating thoracic dystrophy 3, Osteogenesis imperfecta 2, Thanatophoric dwarfism 2 , Campomelic dysplasia 2 , Unspecified skeletal dysplasia 2, Caffey disease 1, Diastrophic dysplasia 1, Fibrochondrogenesis 1, Isolated absent right fibula and short tibia 1 and Asymmetrical focal femoral hypoplasia 1).
- Chitty et al [51] analyzed a population of 26 fetuses with achondroplasia and found that FL was usually below the 3rd centile by 25 weeks' gestation, and always below the 3rd by 30 weeks.
- Arahori et al [52] found 16 cases of skeletal dysplasia (osteogenesis imperfecta, 6; achondroplasia, 3; hypophosphatasia, 2; thanatophoric dysplasia, 1; short rib dysplasia, 1; Ellis–van Creveld syndrome, 1; hypochondroplasia, 1; chondrodysplasia punctata, 1,) in a population of 30 fetuses with micromelia. The FL (defined as a percentage of FL against the mean FL for gestational age at examination) was significantly lower in the skeletal dysplasia group than in the remain population.
- Kurtz et al [53] described FL curve in a group of seven fetuses with achondroplasia. In this group of patient femurs were initially normal but failed to maintain their growth rate and became disproportionately shortened.
- Goncalves et al [54] analyzed 127 cases of 17 skeletal dysplasias. Discriminant analysis showed that the FL was the best biometric parameter to distinguish

among the five most common disorders in this series (thanatophoric dysplasia, osteogenesis imperfecta type II, achondrogenesis, achondroplasia and hypochondroplasia).

Short femur length and IUGR/SGA

Our preliminary literature search identified 25 publications. We selected 5 potentially eligible studies [55-59]. We found 2 additional studies that elude first step research but met the review inclusion criteria [60-8]. In total we included 7 qualifying studies in our analysis (Figure F).

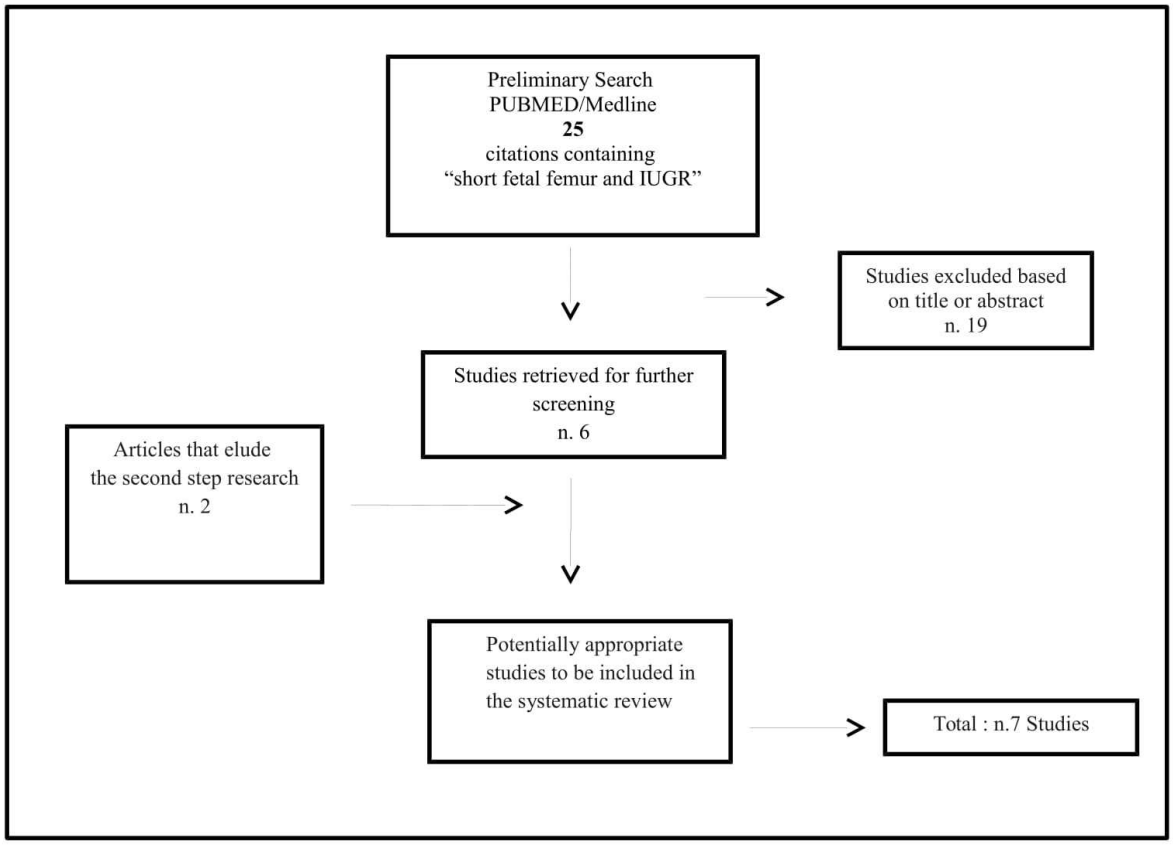


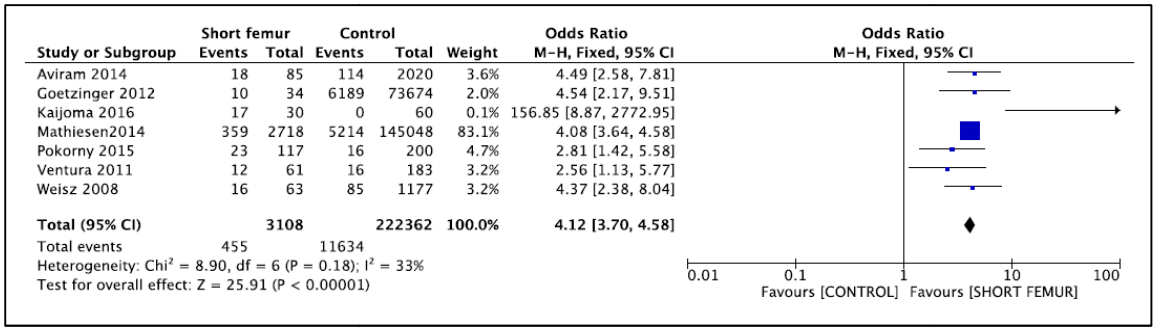
Figure F. Flow chart depicts the search strategy we used to select study for meta-analysis on short fetal femur and IUGR/SGA

In these studies 3108 cases of Short fetal femur (study group) and 222362 normal length fetal femur (control group) were described.

The overall prevalence of IUGR/SGA in the study group was 14,6% (compared with 5.2% in general population).

Overall short fetal femur fetuses were pooled , of which 455 cases presents IUGR/SGA. In control group this sign was present in 11634. The detection rate (DR) and the FPR, for the ability of short FL to detect cases of IUGR/SGA, was reported for each study. Meta-analysis showed a higher incidence of IUGR/SGA in the study group (455/3108, 14,6%) compared with the control group (11634/222362, 5.23%) with an OR of 4.12 (CI 95% 3.70-4.58). The results showed low heterogeneity (33%) [55-60]. (Figure G)

Figure G. Meta-analysis : short femur length and correlation with IUGR/SGA



In addition a higher incidence of perinatal complication were found in fetuses with short femur length:

- low birth weight (study group: 22,10% 72/326, versus control group: 8,57% 307/3580) [55,56,58,59] (Figure H);
- Apgar<7 at 5 minutes (study group: 3,99% 13/326, versus control group: 1,79% 64/3580) [55,56,58,59] (Figure I);

- Preterm delivery (study group: 12,16% 367/3017, versus control group: 8,16% 18140/222119) [55-57,59] (Figure J);
- Fetal demise (study group: 1,91% 55/2867, versus control group: 0,42% 962/220802) [56,57,60] (Figure K);
- No differences were found with regard NICU admission (study group: 31/202, 15.34% vs control group: 329/2220, 14.81%) [55,56] (Figure L).
- Neonatal death was observed only in five pregnancies in the study group, no cases were registered in control group (study group: 2,81% 5/178, versus control group: 0% 0/3257) [56,59,60] (Figure M).

A correlation with hypertensive disorder and short FL was showed by meta-analysis (study group: 15% 41/273, versus control group: 8% 6067/75314) [55,56,58-60] .

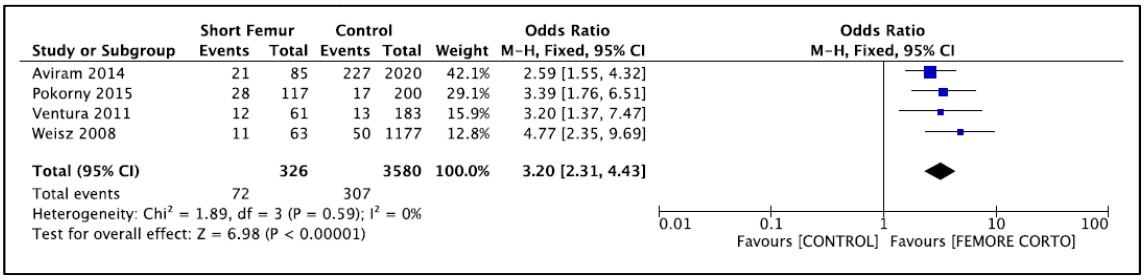


Figure H. Meta-analysis : short femur length and correlation with Low Birth Weight

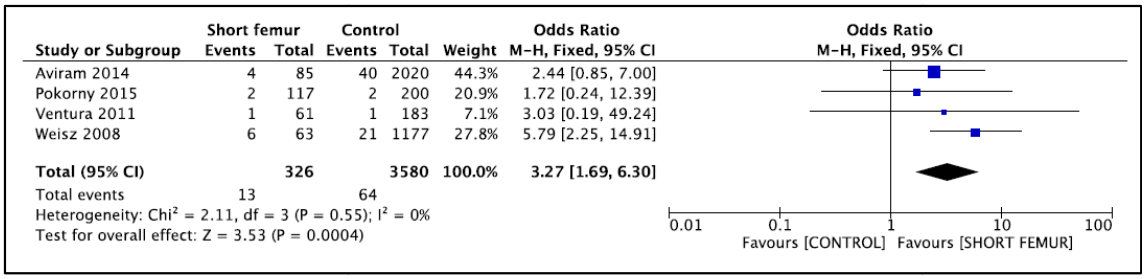


Figure I. Meta-analysis : short femur length and correlation with Apgar-Score < 7 at 5 minutes

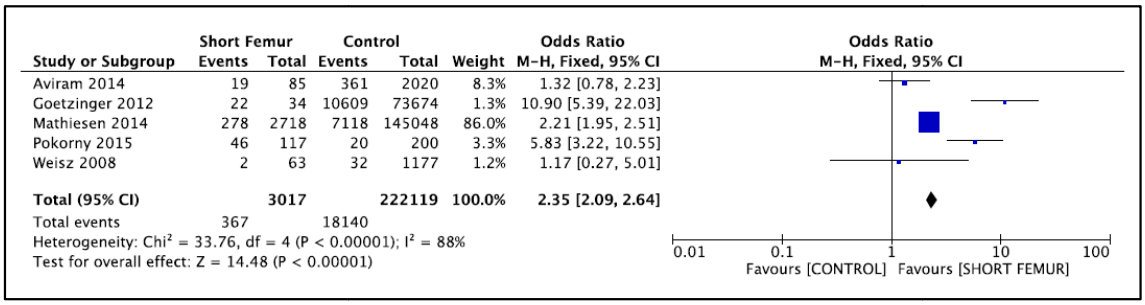


Figure J. Meta-analysis : short femur length and correlation with Preterm Birth

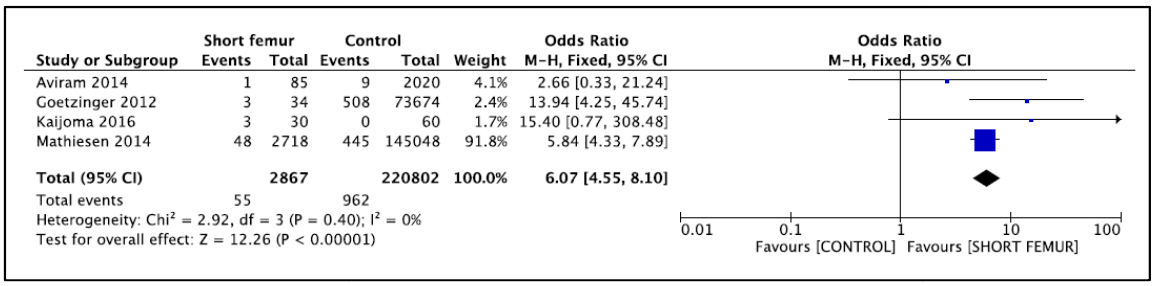


Figure K. Meta-analysis : short femur length and correlation with intrauterine death.

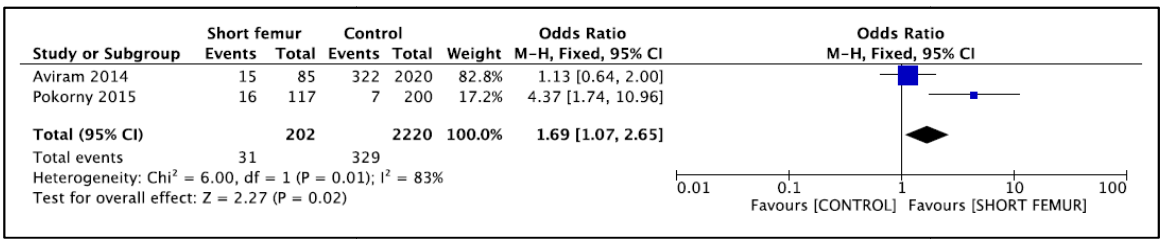


Figure L. Meta-analysis : short femur length and correlation with NICU

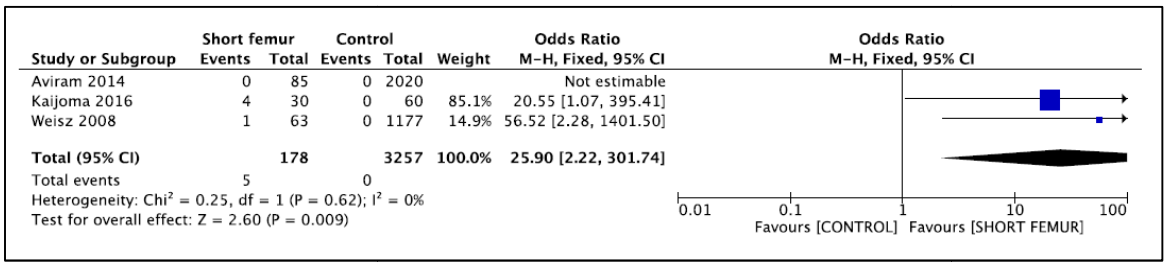


Figure M. Meta-analysis : short femur length and correlation with neonatal death

DISCUSSION

The diagnosis of a short FL occurs in about 5% of pregnancies. It could be associated to other skeletal and structural fetal malformation in the context of a syndromic disease. Anyway it sometime appears as an isolated finding generating anxiety in parents. The detection of a fetal FL below the expected value (< 5th percentile) is a diagnostic challenge for clinicians, with a difficult counseling due to different possible diagnosis: it may be a marker of aneuploidy or associated with other genetic abnormalities or skeletal dysplasia [8-54]. More recent studies [55-60] have suggested as an isolated short femur, in the second trimester of pregnancy, could be an early marker of IUGR and SGA neonate. Short FL may also derived by an inaccurate biometry or may be a variant of normal, especially if present as an isolated finding. The counseling in presence of this marker could be more difficult when it appears later in gestation. For this reason we conducted an analysis of all cases of short FL diagnosed in our institution during every weeks of pregnancy. To our knowledge this is the first study that compares outcomes between two groups of pregnancy with short FL: those with an early diagnosis, before 24 weeks of gestation (group A) respect to fetuses were this sign appear later in gestation (> 25 weeks) (group B). Our results show that a short FL diagnosed ultrasonography before 24 weeks is often linked to other fetal structural anomalies or to aneuploidies, while a diagnosis made later (above 25 weeks) is strongly

associated with IUGR/SGA. In fact non isolated short femur was present in 60,6% of cases before 24 weeks and only in 14,8% of cases after 25 weeks. Conversely, isolated short femur was found in 79% of cases after 25 weeks and only in 33% of cases below 24 weeks. Furthermore, the 27% of this group had abnormal karyotype at the invasive prenatal diagnosis. In group B only 3.7% fetuses presented aneuploidies. On the basis of our data a diagnosis of a short femur earlier in gestation is associated with poor pregnancy outcomes, especially when the sign is not isolated. Infact the group A accounted for only 23 (35%) live born fetuses versus 97,6% of group B. An high percentage of fetal demise and medical termination of pregnancy due to severe fetal anomalies were registered in this group. In our study, according to literature, the presence of a short femur is associated to low birth weight and height. Anyway no difference in perinatal outcome was seen in the two groups. This result is probably due to the high rate of IUGR fetuses in the group B (44,4%) that results in many premature births and neonatal complications. An higher rate of cesarean section were registered in our population compared to vaginal delivery, but mode of delivery were similar in the two groups. An association between isolated short femur and an increased risk of pre-eclampsia was not observed. Results of our study indicate the need for a different counselling and management in pregnancies with a short FL based on gestational age at the diagnosis.

When an early diagnosis is made, it is necessary to inform the couple of the frequent associations with structural and chromosomal defects and to offer them genetic consultation, accurate anomaly scan to exclude other malformations, and if necessary, further investigations like fetal echocardiography and MRI.

A late diagnosis of short FL is associated with a higher percentage of IUGR while the risk of structural and chromosomal anomalies decreases. Therefore women must be informed of the associated risks with intrauterine death and preterm birth, and prematurity complications. In our counseling we should not forget that cases of isolated short FL could have a good prognosis.

We suggest to all sonographers to be cautious when a short femur is found, because potential sources of errors in measurement must be considered. It is important to pay attention to the transducer inclination angle: if the femur is not perpendicular to the probe, the measurement is taken in an oblique plane with a difference in biometry that could vary from 4-10 mm [3]. It is also possible that one end of the bone is shadowed by another bony fetal segment [61]. It is also careful to exclude error in pregnancy dating by checking measurement at first trimester ultrasound. If a short femur is confirmed check for other structural malformation by a segmental and detailed scan of all fetal anatomic district. It is useful to acquire biometry of all long bone to exclude skeletal dysplasia. If isolated bilateral short femur are found, considered initially more common etiology, including ethnic variation, SGA or fetal growth restriction. Assessment of fetal and maternal Doppler measurement and evaluation of amniotic fluid index is essential for the diagnosis of IUGR fetuses. If no alteration in Doppler Velocimetry is found an evaluation of fetal biometry is recommended every two weeks.

For the secondary aim of our research we reviewed the literature and used meta-analytic technique to estimate accuracy of this marker in the prediction of Down Syndrome, IUGR and skeletal dysplasia. Correlation with poor perinatal outcome was also evaluated. The role of short FL in screening of Down Syndrome reveals different results in international literature. In our study the percentage of this genetic disease was 6,8 %.

Todros et al affirmed that short FL is predictive of chromosomal anomalies, but only in fetuses with structural malformations [62]. SOGC practical guideline recommend to consider femur shortening like an ultrasound marker for trisomy 21 with a sensitivity of 16% and FPR of 4% [63]. Our meta-analysis confirms a good performance of this sonographic markers in finding Down Syndrome fetuses, especially in second trimester ultrasound with an odds ratio of 5.12. Anyway the studies included showed great heterogeneity in number of sample, type of study and type of selected population. For this reason we decided to stratified the analysis for high-risk and low risk pregnancy. This analysis showed that femur shortness is more associated to Down syndrome in both population in comparison to euploid fetus. Anyway the association is stronger for high risk group compared to low risk with a difference statistically significant.

A meta-analysis on the association of skeletal dysplasia and short femur failed for the absence of eligible studies. Literature review agreed that a femur >4 SDs below the mean is highly specific for a skeletal dysplasia, likely lethal (assuming correct pregnancy dating).

The diagnosis of a skeletal dysplasia should be considered when limb shortening is detected early and is relatively severe, falling at least two SDs below the mean relative to the biparietal diameter; however, this alone is not diagnostic for a skeletal dysplasia. If during follow-up, the FL is more than 5 mm below 2 SDs (equivalent to greater than 4 SDs below the mean), the sonologist can be certain he or she is dealing with a significant skeletal dysplasia. Kurtz et al. [53] looked at 27 fetuses with shortened femurs and compared FL with biparietal diameter to determine degree of femoral shortening, thus correcting for discrepancies in gestational dating . Of the 12 fetuses with femoral shortening greater than 5 mm below 2 SDs, all 12 had a skeletal dysplasia,

8 of which were lethal. The long bones in all of the extremities should be measured. If limb shortening is present, the segments involved should be defined. A detailed examination of the involved bones is necessary to exclude absence, hypoplasia, and malformation of the bones. The bones should be assessed for presence, curvature, degree of mineralization, and fractures. The femur length–abdominal circumference ratio (<0.16 suggests lung hypoplasia) and femur length–foot length ratio (normal = 1, <1 suggests skeletal dysplasia) should be calculated.

Non isolated short FL rate diverges in literature data: Vermeer et al. and Papageorghiou et al. reported 22% and 36% cases respectively associated with chromosomal or structural abnormalities [50]; Todros et al. described 46% of non isolated short FL cases [62]. While counseling and management of fetuses with not isolated short femur is more clear to clinicians and consist in genetic counseling and prenatal diagnosis for fetal Karyotype, the finding of an isolated short femur could be of more difficult management. In our study 58% of cases presented an isolated short FL. In others studies, this percentage was 83% et 78% [50]. Recent literature highlights the relationship between short femur and IUGR (40% in Papageorghiou, 39% in Todros and 43% in Vermeer). [50,62]. In our population we found a percentage of 33%, with a major prevalence in group B (44,4. % vs 19%). Our meta-analytic data demonstrated that an isolated short femur increase IUGR/SGA risk four-fold respect to fetuses with a normal fetal femur biometry [55-60].

In terms of aetiology of femur shortening in case of SGA/IUGR fetuses, literature data suggests different mechanisms. Cases due to placental insufficiency might be explained by redistribution of blood flow, with increased flow to the heart and brain and decreased flow to the lower body. In fact reduced FL is concordant with the small AC [59]. Zalel

et al suggested that the bone undergrowth was due to altered secretion of fibroblast growth factor 2 by the abnormal placenta [55,62]. Additionally, in others studies, PAPP-A is a promising prognostic parameter for the FL in the first and second trimester [55].

Another aim of the present study was to investigate the different associations with adverse perinatal outcomes and isolated short femur length. The meta-analysis showed an increased risk for preterm birth (OR 2.35), fetal demise (OR 6.07), Apgar <7 at 5 minutes (OR 3.27). This data asserts that obstetricians' attitude in the management of these fetuses could be careful to prevent adverse pregnancy outcome. For this reason it is suitable to follow these patients in fetal medicine units and pregnancy high risk section [55-60].

CONCLUSION

The diagnosis of short FL is often a challenge in obstetrics. The results of our study could help clinicians in counseling these patients in presence of this ultrasound finding. The diagnosis of a non isolated short FL before 24 weeks of gestation is associated to poor pregnancy outcome. When a short femur arise late in gestation and in isolated form, pregnancy outcome is better in term of chromosomal abnormalities but high rate of IUGR, SGA and neonatal complication is possible. We conclude proposing a diagnostic algorithm (Figure N).

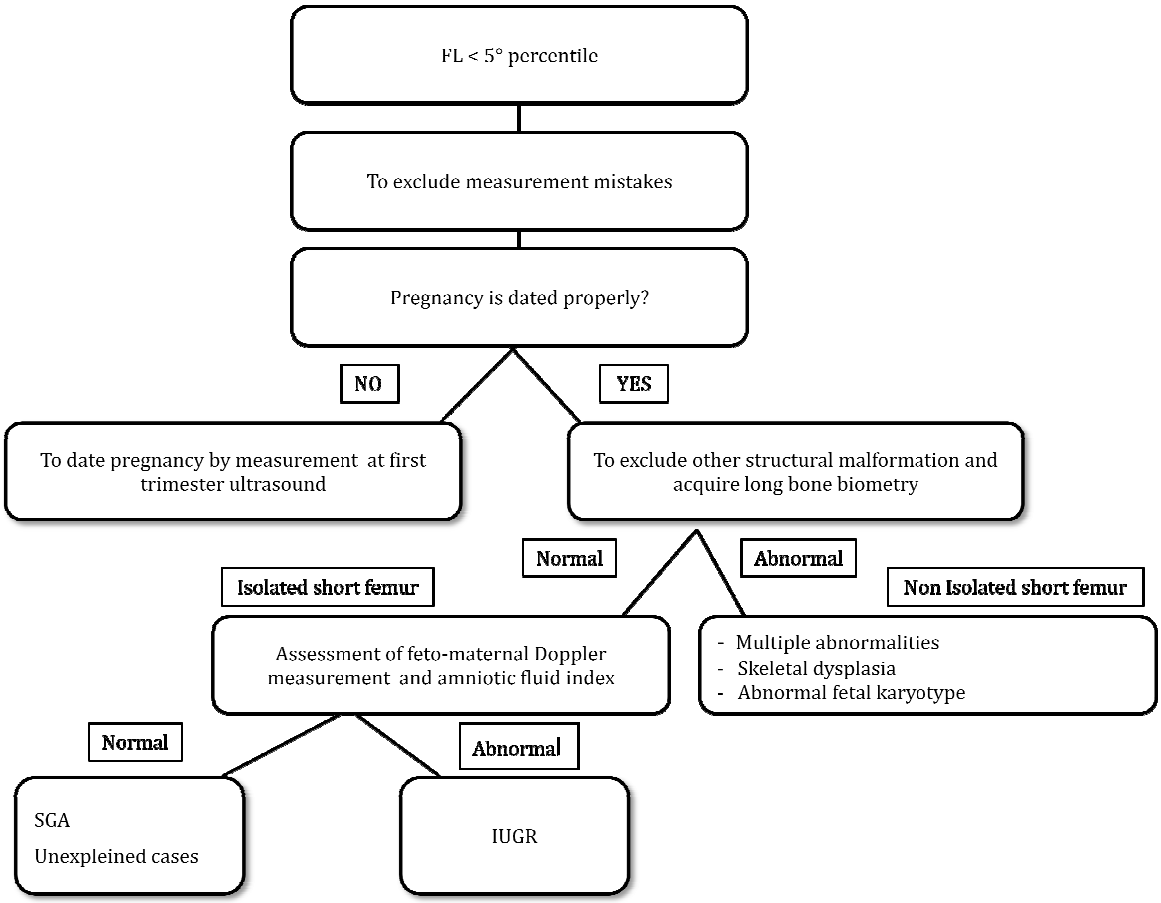
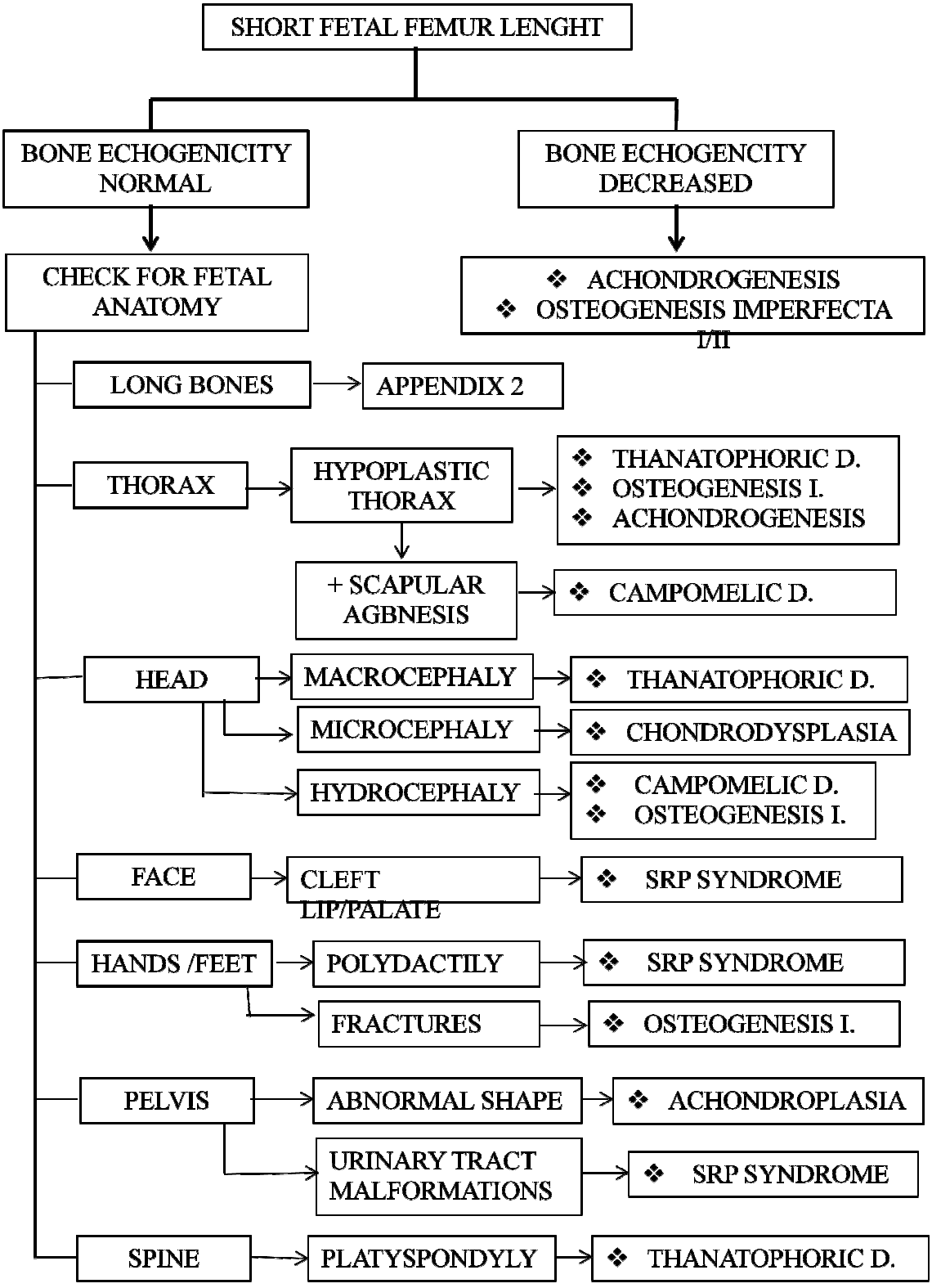
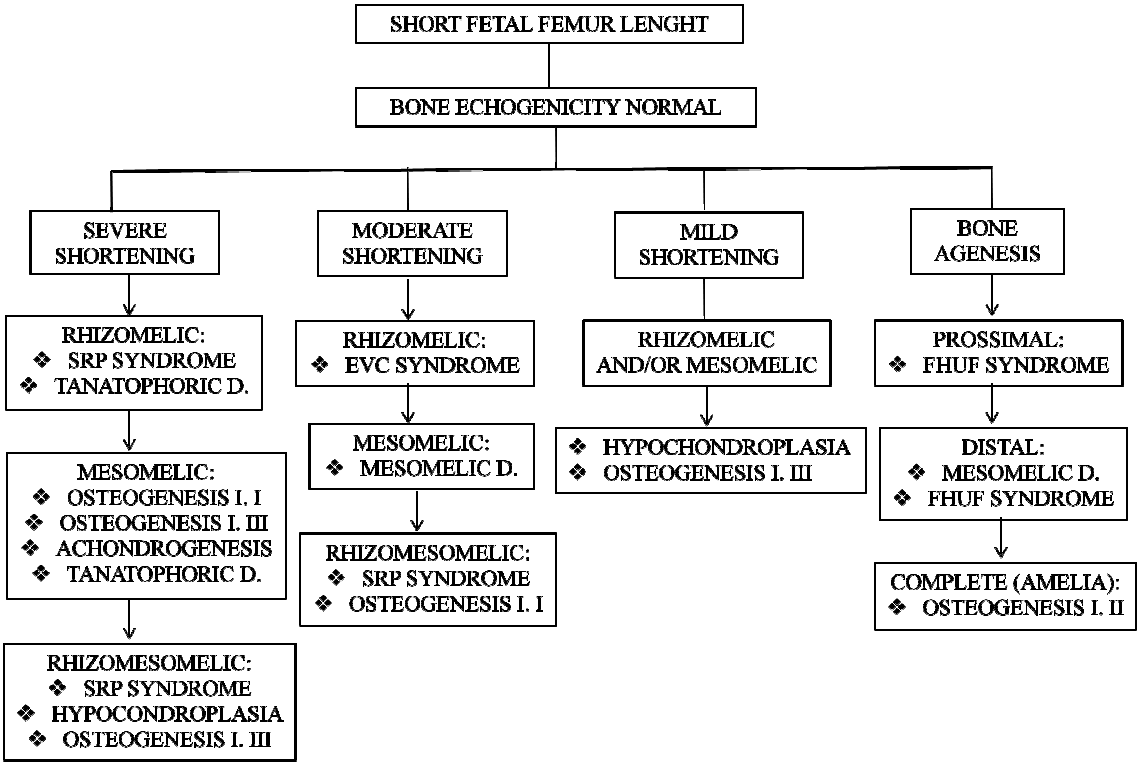


Figure N. Diagnostic flow-chart for prenatal diagnosis of short femur lenght

Appendix 1. Short femur length and Skeletal Dysplasia



Appendix 2. Short femur length and Skeletal Dysplasia



Appendix 3. ABBREVIATIONS

(2D) two - dimensional
(3D) three - dimensional
(4D) four-dimensional
(NT) nuchal translucency thickness
(CRL) Crown-rump-length
(DRs) Detection rates
(TOP) termination of pregnancy
(FPR) false positive rate
(free-ßhCG) free beta human chorionic gonadotropin
(PAPP-A) pregnancy-associated plasma protein-A
(cfDNA) cell-free DNA
(NIPT) non-invasive prenatal testing
(PPVs) positive predictive values
(CVS) Chorionic villus sampling
(MRI) Magnetic resonance imaging
(CNS) central nervous system
(AER) apical ectodermal ridge
(FL) femur length
(SIEOG) Italian ultrasound Society of Obstetrics and Gynecology
(SD) standard deviations
(BPD) biparietal diameter
(FPR) false-positive rate
(HL) humeral length
(CPC) choroid plexus cyst
(EIF) echogenic intracardiac focus
(SUA) Single umbilical artery
(LR) likelihood ratios
(NF) nuchal fold
(US) ultrasound
(NICU) Neonatal Intensive Care Unit

REFERENCES

Introduction
1. Prenatal diagnosis: state of art

1. Delhanty JDA, Handyside AH. The origin of genetic defects in the human and their detection in the preimplantation embryo. Human Reproduction Update. 1995;1:201–215.
2. Dolk H, Loane M, Garne E. The prevalence of congenital anomalies in Europe. Adv Exp Med Biol. 2010;686:349-64.
3. Moore KL, Persaud TVN. Human birth defects. In The Developing Human: Clinically Orientated Embryology. Philadelphia: Saunders; 2003:157–186.
4. Benson CB, Doubilet PM. The history of imaging in obstetrics. Radiology. 2014;273(2suppl):S92–S110.
5.Theodore J, Dubinsky MD. Advances in Obstetric Ultrasound. Philadelphia, PA: W.B.Saunders Company;2011.
6. Rayburn WF, Jolley JA, Simpson LL. Advances in ultrasound imaging for congenital malformations during early gestation. Birth Defects Res A Clin Mol Teratol. 2015 Apr;103(4):260-8.
7. Merz E, Abramowicz JS. 3D/4D ultrasound in prenatal diagnosis: is it time for routine use? Clin Obstet Gynecol. 2012 Mar;55(1):336-51.
8. SIEOG Guidelines 2015.
9. Practice Guidelines: performance of first-trimester fetal ultrasound scan (UOG Volume 41, Issue 1, Date: January 2013, Pages: 102-113
10. Practice Guidelines for performance of the routine mid-trimester fetal ultrasound scan (UOG Volume 37, Issue 1, Date: January 2011, Pages: 116 - 126)
11. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol. 2004;191(1):45–67.
12. Karim JN, Roberts NW, Salomon LJ, et al. Systematic review of first trimester ultrasound screening for detection of fetal structural anomalies and factors that affect screening performance. Ultrasound Obstet Gynecol. 2016 Aug 22.
13. Crane JP, Le Fevre ML, Winborn RC, et al. A randomized trial of prenatal ultrasonographic screening: impact on the detection, management, and outcome of anomalous fetuses. The RADIUS Study Group. Am J Obstet Gynecol. 1994;171(2):392–399.
14. Grandjean H, Larroque D, Levi S. The performance of routine ultrasonographic screening of pregnancies in the Eurofetus Study. Am J Obstet Gynecol. 1999;181(2):446–454.
15. Eurocat Perinatal Health Report 2013.
16. Le Ray C, Lacerte M, Iglesias MH, Audibert F, Morin L. Routine third trimester ultrasound: what is the evidence? J Obstet Gynaecol Can. 2008 Feb;30(2):118-22.
17. Bawa M, Kannan NL. Even a single third trimester antenatal fetal screening for congenital anomalies can be life saving. Indian J Pediatr. 2010 Jan;77(1):103-4.
18. Carles D, Pelluard F, Alberti EM, Gangbo F. Fetopathological examination and congenital anomalies in the third trimester. Bull Acad Natl Med. 2005 Nov;189(8):1789-99; discussion 1799-801.
19. Benn P, Cuckle H, Pergament E. Non-invasive prenatal testing for aneuploidy: current status and future prospects. Ultrasound Obstet Gynecol. 2013 Jul;42(1):15-33.
20.Ghaffari SR, Tahmasebpour AR, Jamal A, el al. First-trimester screening for chromosomal abnormalities by integrated application of nuchal translucency, nasal bone, tricuspid regurgitation and ductus venosus flow combined with maternal serum free β-hCG and PAPP-A: a 5-year prospective study. Ultrasound Obstet Gynecol. 2012 May; 39(5):528-34.
21.Ferguson-Smith MA, Yates JRW. Maternal age specific rates for chromosome aberrations and factors influencing them: report of a collaborative European study on 52965 amniocenteses. Prenat Diagn 1984; 4 (Spec No): 5–44.

22.Wald NJ, Cuckle HS, Densem JW, et al. Maternal serum screening for Down's syndrome in early pregnancy. BMJ 1988; 297: 883–887.
23.Wald NJ, Rodeck C, Hackshaw A, et al. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). Health Technol Assess 2003
24.Benn PA, Egan JF, Fang M, et al. Changes in the utilization of prenatal diagnosis. Obstet Gynecol 2004; 103: 1255–1260.
25. Kagan KO, Wright D, Baker A, et al. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 2008; 31: 618–624.
26. Maiz N, Valencia C, Kagan KO, et al. Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation. Ultrasound Obstet Gynecol 2009; 33: 512–517.
27. Kagan KO, Valencia C, Livanos P, et al. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11+0 to 13+6weeks of gestation. Ultrasound Obstet Gynecol 2009; 33: 18–22.
28.Kagan KO, Cicero S, Staboulidou I, et al. Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation. Ultrasound Obstet Gynecol 2009; 33: 259–264.
29. Gil MM, Akolekar R, Quezada MS, et al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. Fetal Diagn Ther 2014; 35: 156–173.
30. Gil MM, Accurti V, Santacruz B, et al. Analysis of cellfree DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol. 2017; Apr 11. [Epub ahead of print]
31. Guex N, Iseli C, Syngelaki A et al. A robust second generation genome wide test for fetal aneuploidy based on shotgun sequencing cellfree DNA in maternal blood. Prenatal diagnosis 2013: 33:707-710.
32. Rose NC, Benn P, Milunsky A. Current controversies in prenatal diagnosis 1: should NIPT routinely include microdeletions/microduplications? Prenatal diagnosis 2016: 36: 10-14.
33. Colosi E, D'Ambrosio V, Periti E. First trimester contingent screening for trisomies 21,18,13: is this model cost efficient and feasible in public health system?. J Matern Fetal Neonatal Med. 2017 Jan 4:1-6.
34. Steele MW, Breg WR. Chromosome analysis of human amniotic fluid cells. Lancet. 1966;1:383–385.
35. Jenkins TM, Wapner RJ. First trimester prenatal diagnosis: chorionic villus sampling. Semin Perinatol. 1999; 23:403–413.
36. Royal College of Obsterics and Gyecology (RCOG) Green-top Guideline No. 8: Amniocentesis and Chorionic Villus Sampling.
37. Douglas Wilson R. Amniocentesis and chorionic villus sampling. Curr Opin Obstet Gynecol. 2000;12:81–86.
38. Hardisty EE, Vora NL. Advances in genetic prenatal diagnosis and screening. Curr Opin Pediatr. 2014 Dec; 26(6):634-8.
39. Mi-Young L, Hye-Sung W. Technique of fetal echocardiography. Obstet Gynecol Sci. 2013 Jul; 56(4): 217–226.
40. AIUM. Practice Parameter for the Performance of fetal echocardiography. 2013.
41. Pugash D, Bruggerb PC, Bettelheimc D, et al. Prenatal ultrasound and fetal MRI: the comparative value of each modality in prenatal diagnosis. Eur J Radiol 2008;68:214–226.
42. Kanal E. Pregnancy and the safety of magnetic resonance imaging. Magn Reson Imaging Clin North Am. 1994; 2:309–317.
43. Wendy R. Uhlmann (Editor), Jane L. Schuette (Editor), Beverly Yashar (Editor). A guide to genetic counseling second edition. Wiley- Blackwell.
44. Mark I. Evans Prenatal Diagnosis McGraw Hill Professional, 2006.

2. Prenatal development of human femur

1. Anatomia umana di Frederic H. Martini, Michael J. Timmons, Robert B. Tallitsch
2. Egawa S, Miura S, Yokoyama H, et al. Growth and differentiation of a long bone in limb development, repair and regeneration. Dev Growth Differ. 2014 Jun;56 (5):410-24.
3. Sadler, TW. Langman's Medical Embryology, 11th ed. 2009. Lippincott Williams &Wilkins, Baltimore, Maryland 21201.
4. Shubin NH, Alberch P. A morphogenetic approach to the origin and basic organization of the tetrapod limb. Evol Biol. 1996; 20:319–387.
5. Robertson KE, Tickle C.Recent molecular advances in understanding vertebrate limb development. Br J Plast Surg. 1997; 50:109–115.
6. Niswander L, Jeffrey S, Martin GR, et al. A positive feedback loop coordinates growth and patterning in the vertebrate limb. Nature. 1994; 317:609–612.
7. Crossley PH, Minowada G, MacArthur CA, et al. Roles for FGF8 in the induction, initiation, and maintenance of chick limb development. Cell. 1996; 84:127–136.
8. Niswander L, Tickle C, Vogel A, et al. FGF-4 replaces theapical ectodermal ridge and directs outgrowth and patterning of the limb. Cell 1994;75: 579–587.
9. Zuzarte-Luis V, Hurle JM. Programmed cell death in the developing limb. Int. J. Dev. Biol. 2002; 46, 871–876.
10. Erlebacher A, Filvaroff EH, Gitelman SE, et al. Toward a molecular understanding of skeletal development. Cell. 1995; 80: 371–378.
11. Williams, GM, Klisch, SM, Sah, RL. Bioengineering cartilage growth, maturation, and form. Pediatr. Res. 1196; 63: 527–534.
12. Putz R. Development and growth of the epiphysis. *Z* Orthop Ihre Grenzgeb. 1996; 134: 391-395.
13. Mackie EJ, Ahmed YA, Tatarczuch L, et al. Endochondral ossification: how cartilage is converted into bone in the developing skeleton. Int J Biochem Cell Biol. 2008; 40: 46-62.
14. Johnson RL, Tabin CJ. Molecular models for vertebrate limb development. Cell. 1997; 90:979–990.
15. Friedenstein AJ. Osteogenic stem cells in the bone marrow. In: Heersche JNM, Kanis JA (eds.) Bone Miner Research. Elsevier Science Publishers, Amsterdam, The Netherlands,1990. pp. 243–270.
16. Ducy P, Zhang R, Geoffroy V, et al. Osf2/Cbfa1: A transcriptional activator of osteoblast differentiation. Cell. 1997; 89:747–754.
17. Otto F, Thornell AP, Crompton T, et al. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell. 1997; 89: 765–771.
18. Komori T, Yagi H, Nomura S, et al. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell. 1997; 89:755–764.
19. Nakashima K, Zhou X, Kunkel G, et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell. 2002; 108:17–29.
20. Campagnoli C, Roberts IA, Kumar S, et al. Identification of mesenchymal stem/progenitor cells in human first trimester fetal blood, liver, and bone marrow. Blood. 2001; 98:2396–2402.
21 Schipani E, Ryan HE, Didrickson S, et al. Hypoxia in cartilage: HIF-1alpha is essential for chondrocyte growth arrest and survival. Genes Dev. 2001; 15:2865–2876.
22. Zelzer E, Mamluk R, Ferrara N, et al. VEGFA is necessary for chondrocyte survival during bone development. Development. 2004; 131:2161–2171.
23 . Zelzer E, Glotzer DJ, Hartmann C, et al. Tissue specific regulation of VEGF expression by Cbfa1/Runx2 during bone development. Mech Dev. 2001; 106:97–106.
24. Babarina AV, Mollers U, Bittner K, et al. Role of the subchondral vascular system in endochondral ossification: endothelial cell-derived proteinases depress late cartilage differentiation in vitro. Matrix Biol. 2001; 20: 205-213.

25. Higashikawa A, Kawaguchi H, Nakamura K, et al. Interactions of chondrocytes and osteoblasts during endochondral bone formation. 2006; 16: 829-836.
26. SIEOG Guidelines 2015.
27. ISUOG. Practice guidelines for performance of the routine mid-trimester fetal ultrasound scan. 2010.
28. O'Brien GD, Queenan JT, Campbell S. Assessment of gestational age in the second trimester by real-time ultrasound measurement of the femur length. Am J Obstet Gynecol 1981;139:540-5.
29. Adiri CO, Anyanwu GE, Agwuna KK, et al. Use of fetal biometry in the assessment of gestational age in South East Nigeria: Femur length and biparietal diameter. Niger J Clin Pract. 2015;18:477-82.
30. Chitty LS, Altman DG, Henderson A, et al. Charts of fetal size: 4. Femur length.Br J Obstet Gynaecol. 1994 Feb;101(2):132-5.
31. Chitty LS, Altman DG. Charts of fetal size: limb bones. BJOG. 2002 Aug;109(8):919-29.
32. Shipp T, Bromley B, Mascola M, et al. Variation in fetal femur length with respect to maternal race. J Ultrasound Med 2001; 20:141-4.
33. Ghi T, Cariello L, Rizzo L, et al. Customized Fetal Growth Charts for Parents' Characteristics, Race, and Parity by Quantile Regression Analysis: A Cross-sectional Multicenter Italian Study. J Ultrasound Med. 2016 Jan;35(1):83-92.

3. Fetal short femur lenght: definition and implications

3.1 Short femur length and aneuploidies

1.Papageorghiou AT, Fratelli N, Leslie k, et al. Outcome of fetuses with antenatally diagnosed short femur. Ultrasound Obstet Gynecol. 2008 May; 31(5):507-11. doi: 10.1002/uog.5265.
2. Harper LM, Gray D, Dicke J, et al. Do race-specific definitions of short long bones improve the detection of down syndrome on second-trimester genetic sonograms? J Ultrasound Med. 2010 Feb;29(2):231-5.
3 Dicke JM, Gray DL, Songster GS, et al. Fetal biometry as a screening tool for the detection of chromosomally abnormal pregnancies. Obstet Gynecol 1989; 74:726– 729.
4. Benacerraf BR, Gelman R, Frigoletto FD. Sonographic identification of second-trimester fetuses with Down's syndrome. N Engl J Med. 1987; 317: 1371–1376.
5. Nyberg DA, Resta RG, Luthy DA, et al. Humerus and femur length shortening in the detection of Down's syndrome. Am J Obstet Gynecol. 1993; 168:534– 538.
6. SIEOG Guidelines 2015.
7. ISUOG. Practice guidelines for performance of the routine mid-trimester fetal ultrasound scan. 2010.
8. Hadlock FP, Harrist RB, Sharman RS, et al. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gynecol. 1985 Feb 1;151(3):333.
9. Weisz B., David A.L., Chitty L., et al. Association of isolated short femur in the mid-trimester fetus with perinatal outcome, Ultrasound Obstet. Gynecol. 2008; 31:512–516.
10. Mailath-Pokorny M., Polterauer S., Worda K., et al. Isolated short fetal femur length in the second trimester and the association with adverse perinatal outcome: Experiences from a Tertiary Referral Center. Plos One. 2015 Jun 5;10(6):e0128820.
11. Özlü T., Ozcan T., Fetal isolated short femur in the second trimester and adverse pregnancy outcomes, Prenat. Diagn. 2013; 33:1063–1069.
12. Baird PA, Anderson TW, Newcombe HB, et al. Genetic disorders in children and young adults: a population study. Am J Human Genet. 1988; 42 (5): 677–693.
13. Sprigg A. Fetalmalformations diagnosed antenatally 1: general principles. Br J Hosp Med. 1995; 54 (8): 387–390.

14. Jones KL. Smith's recognizable patterns of human malformation. 6th ed. Philadelphia. Elsevier Saunders. 2006.
15. Down JLH. Observations on an ethnic classification of idiots. London Hosp Clin Lect Rep 1866; 3: 259.
16. Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatiques de neuf enfants mongoliens. C R Acad Sci 1959; 248: 1721-1722.
17. Pavarino Bertelli EC, Biselli JM, et al. Clinical profile of children with Down syndrome treated in a genetics outpatient service in the southeast of Brazil. Rev Assoc Med Bras. 2009 Sep-Oct;55(5):547-52.
18. Benhaourech S, Drighil A, Hammiri AE. Congenital heart disease and Down syndrome: various aspects of a confirmed association. Cardiovasc J Afr. 2016 Sep/Oct;27(5):287-290.
19. Down Syndrome. Orphanet http://www.orpha.net/consor/cgi-bin/OC_Exp.php?Expert=870
20. Kagan KO, Wright D, Baker A, et al. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 2008; 31: 618–624.
21. Maiz N, Valencia C, Kagan KO, et al. Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation. Ultrasound Obstet Gynecol 2009; 33: 512–517.
22. Kagan KO, Valencia C, Livanos P, et al. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11+0 to 13+6weeks of gestation. Ultrasound Obstet Gynecol 2009; 33: 18–22.
23. Kagan KO, Cicero S, Staboulidou I,et al. Fetal nasal bone in screening for trisomies 21, 18 and 13 and
Turner syndrome at 11–13 weeks of gestation. Ultrasound Obstet Gynecol 2009; 33: 259–264.
24. Maiz N, Wright D, Ferreira AFA, et al. Amixture model of ductus venosus pulsatility index in screening for aneuploidies at 11–13 weeks' gestation. Fetal Diagn Ther 2012; 31: 221–229.
25. Gil MM, Akolekar R, Quezada MS, et al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. Fetal Diagn Ther 2014; 35: 156–173.
26. Van den Hof MC, Wilson RD, et al. Fetal soft markers in obstetric ultrasound. J Obstet Gynaecol Can. 2005 Jun;27(6):592-636.
27. Rao R, Platt LD. Ultrasound screening: Status of markers and efficacy of screening for structural abnormalities. Semin Perinatol. 2016 Feb; 40(1):67-78.
28. Rumi Kataguiri M, Araujo Júnior E, Silva Bussamra LC, et al. Influence of second-trimester ultrasound markers for Down syndrome in pregnant women of advanced maternal age. J Pregnancy. 2014;2014:785730.
29. Lockwood C, Benacerraf B, Krinsky A, et al. A sonographic screening method for Down syndrome. Am J Obstet Gynecol. 1987 Oct;157(4 Pt 1):803-8.
30. Brumfield CG, Hauth JC, Cloud GA, et al. Sonographic measurements and ratios in fetuses with Down syndrome. Obstet Gynecol. 1989; 73: 644–646.
31. Dicke JM, Gray DL, Songster GS, et al. 1989. Fetal biometry as a screening tool for the detection of chromosomally abnormal pregnancies. Obstet Gynecol 74: 726–729.
32. Grist TM, Fuller RW, Albiez KL, et al. Femur length in the US prediction of trisomy 21 and other chromosomal abnormalities. Radiology. 1990; 174: 837–839.
33. Schluter PJ, Pritchard G. Mid trimester sonographic findings for the prediction of Down syndrome in a sonographically screened population. Am J Obstet Gynecol. 2005; 192: 10–16.
34. Malone F, Nyberg D, Vidaver J, et al. First and second trimester evaluation of risk (FASTER) trial: The role of second trimester Obstet Gynecol. 2007 Jul;110(1):10-7.
35. Smith-Bindman R, Chu P, Goldberg J. Second trimester prenatal ultrasound for the detection of pregnancies at increased risk of Down syndrome. Prenat Diagn. 2007; 27: 535–544.
36. Benn P, Egan JF. Second trimester prenatal ultrasound and screening for Down syndrome. Prenat Diagn. 2007; 27: 884.

37. Vintzileos AM, Egan JF. Adjusting the risk for trisomy 21 on the basis of second-trimester ultrasonography. Am J Obstet Gynecol. 1995; 172: 837–844.
38. Smith-Bindman R, Hosmer W, Feldstein VA, et al. Second-trimester ultrasound to detect fetuses with Down syndrome: a meta-analysis. JAMA 2001; 285: 1044–1055.
39. Agathokleous M, Chaveeva P, Poon LC, et al. Meta-analysis of second-trimester markers for trisomy 21. Ultrasound Obstet Gynecol. 2013 Mar;41(3):247-61.
40. Perni SC, Chervenak FA, Kalish RB, et al. Intraobserver and interobserver reproducibility of fetal biometry. Ultrasound Obstet Gynecol. 2004 Nov;24(6):654-8.
41. Gray DL, Dicke JM, Dickerson R, et al. Reevaluating humeral length for the detection of fetal trisomy 21. J Ultrasound Med. 2009 Oct;28(10):1325-30.
42. Di Pietro JA, CostiganKA, CristofaloEA, et al. Choroid plexus cysts do not affect fetal neurodevelopment. J Perinatol. 2006;26 (10):622–627.
43. Twining P, Zuccollo J, Clewes J, et al. Fetal choroid plexus cysts: a prospective study and review of the literature. Br J Radiol. 1991;64(758):98–102.
44. Snijders RJ, Shawa L, NicolaidesKH. Fetal choroid plexus cysts and trisomy18: assessment of risk based on ultrasound findings and maternal age. Prenat Diagn. 1994;14(12):1119–1127.
45. Petrikovsky BM, Challenger M, Wyse LJ. Natural history of echogenic foci within ventricles of the fetal heart. Ultrasound Obstet Gynecol 1995; 5(2): 92–4.
46. Lim KI, Austin S, Wilson RD. Echogenic intracardiac foci: incidence laterality, and association with Down syndrome: a prospective study. J Ultrasound Med 1998;17(3): S11.
47. Nguyen HT, Herndon CD, CooperC, et al. The Society for Fetal Urology consensus statement on the evaluation and management of antenatal hydronephrosis. J Pediatr Urol. 2010;6 (3):212–231.
48. Broadley P, McHugo J, Morgan I, et al. The 4 year outcome following the demonstration of bilateral renal pelvic dilatation on prenatal renal ultrasound. Br J Radiol. 1999;72(855):265–270.
49. Benacerraf BR, Mandell J, Estroff JA, et al. Fetal pyelectasis: a possible association with Down syn- drome. Obstet Gynecol. 1990;76(1):58–60.
50. Rembouskos G, Cicero S, LongoD, et al. Single umbilicalarteryat11-14weeks'gestation: relation to chromosomal defects. Ultrasound Obstet Gynecol. 2003; 22 (6):567–570.
51. Sepulveda W, Sebire NJ. Fetal echogenic bowel: a complex scenario. Ultrasound Obstet Gynecol. 2000;16: 510–4.
52. Nyberg DA, Dubinsky T, Resta RG, et al. Echogenic fetal bowel during the second trimester: clinical importance. Radiology 1993;188: 527–31.
53. Crane JP, Gray DL. Sonographically measured nuchal skin fold thickness as a screening tool for Down syndrome: results of a prospective clinical trial. Obstet Gynecol. 1991;77(4):533–536.
54. Nyberg DA, Resta RG, Luthy DA, et al. Prenatal sonographic findings in Down syndrome. Review of 94 cases. Obstet Gynecol 1990;76:370–7.
55. Cicero S, Sonek JD, Mckenna DS, et al. Nasal bone hypoplasia in trisomy 21 at 15–22 weeks' gestation. Ultrasound Obstet Gynecol. 2003; 21: 15–18.

3.2 Short femur length and skeletal dysplasia

1. Bonafe L, Cormier-Daire V, Hall C, et al. Nosology and classification of genetic skeletal disorders: 2015 revision. Am J Med Genet A. 2015 Dec;167A(12):2869-92.
2. Krakow D, Rimoin DL. The skeletal dysplasias. Genet Med. 2010; 12:327–341.
3. Sharony R, Browne C, Lachman RS et al. Prenatal diagnosis of the skeletal dysplasias. Am J Obstet Gynecol. 2013; 169:668–675.
4. Parilla BV, Leeth EA, Kambich MP et al. Antenatal detection of skeletal dysplasias. J Ultrasound Med. 2003; 22: 255–258.

5. Gaffney G, Manning N, Boyd PA et al. Prenatal sonographic diagnosis of skeletal dysplasias–a report of the diagnostic and prognostic accuracy in 35 cases. Prenat Diagn. 1998; 18:357–362.
6. Lachman RS, editor. Taybi and Lachman's radiology of syndromes, metabolic disorders and skeletal dysplasias. Philadelphia: Mosby Elsevier. 2007.
7. Kurtz AB, Needleman L, Wapner RJ, et al. Usefulness of a short femur in the in utero detection of skeletal dysplasias. Radiology 1990; 177:197– 200.

3.3 Short femur length and intrauterine growth restriction

1. Papageorghiou AT, Fratelli N, Leslie K, et al. Outcome of fetuses with antenatally diagnosed short femur. Ultrasound Obstet Gynecol 2008; 31:507–11.
2. Todros T, Massarenti I, Gaglioti P, et al. Fetal short femur length in the second trimester and the outcome of pregnancy. BJOG 2004;111:83–5.
3. Ventura W, Huaman J, Nazario CE, et al. Perinatal outcomes after sonographic detection of isolated short femur in the second trimester.J Clin Ultrasound. 2012;40:63–7.
4. Weisz B, David AL, Chitty L, et al. Association of isolated short femur in the mid-trimester fetus with perinatal outcome. Ultrasound Obstet Gynecol 2008;31:512–6.
5. Giancotti A, Muto B, Spagnuolo A, D'Ambrosio V, et al. "Dallo screening del I trimestre al feto a rischio di IUGR" News & Opinion in Ginecologia Volume 5 pg. 12-18→ che anno?
6.Lausman A, Kingdom J; MATERNAL FETAL MEDICINE COMMITTEE. Intrauterine growth restriction: screening, diagnosis, and management. J Obstet Gynaecol Can. 2013 Aug; 35(8): 741-748.
7. American College of Obstetricians and Gynecologists. ACOG Practice bulletin no. 134: fetal growth restriction. Obstet Gynecol. 2013 May; 121(5): 1122-33.
8. C. Benedetto, P. Sismondi, Ginecologia e ostetricia. Patologia della gravidanza, Ritardo di crescita endouterino (di Ferrazzi E., Stampalija T., Casati D.), pagg. 379-388, Edizioni Minerva Medica, Torino, 2013.
9. Vermeer N., Bekker M.N., Association of isolated short fetal femur with intrauterine growth restriction. Prenat Diagn. 2013; 33:365–370.
10. Qin X, Wergedal JE, Rehage M, et al. Pregnancy associated plasma protein-A increases osteoblast proliferation in vitro and bone formation in vivo. Endocrinology. 2006; 147: 5653–5661.
11. Morales-Roselló J, LLorens NP. Outcome of fetuses with diagnosis of isolated short femur in the second half of pregnancy, ISRN. Obstetrics and Gynecology. 2012. doi:10.5402/2012/268218.

References experimental study

1. Goetzinger KR, Cahill AG, Macones GA, et al. Isolated short femur length on mid-trimester ultrasound: a marker for fetal growth restriction and other adverse perinatal outcomes. J Ultrasound Med. 2012; 31(12):1935–1941
2. De Carvalho AA, Carvalho JA, Figueiredo IJr, et al Association of midtrimester short femur and short humerus with fetal growth restriction. Pren Diagn. 2012; 33(2):130-3
3. Salomon Z, Alfirevic Z, Berghella V, et al. Practise guidelines for performance of the routine mid-trimester fetal ultrasound scan. Ultrasound Obstet Gynecol.2011; Jan; 37(1):116-26
4. SIEOG [internet]. Italy: SIEOG guidelines 2015; [cited 2015 Nov 30]. Avaiable from : http://www.sieog.it/linee-guida/.

5. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gynecol. 1985 Feb 1;151(3):333-7.
6. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and metaanalyses: the PRISMA statement. PLoS.
7. Linee guida AIPE 2013.
8. Mathiesen JM, Aksglaede L, Skibsted L, Petersen OB, Tabor A; Danish Fetal Medicine Study Group. Outcome of fetuses with short femur length detected at second-trimester anomaly scan: a national survey. Ultrasound Obstet Gynecol. 2014 Aug;44(2):160-5. doi: 10.1002/uog.13286. Epub 2014 Jun 27.
9. Rumi Kataguiri M, Araujo Júnior E, Silva Bussamra LC, Nardozza LM, Fernandes Moron A. Influence of second-trimester ultrasound markers for Down syndrome in pregnant women of advanced maternal age. J Pregnancy. 2014;2014:785730. doi: 10.1155/2014/785730. Epub 2014 Mar 25.
10. Shah YG, Eckl CJ, Stinson SK, Woods JR Jr. Biparietal diameter/femur length ratio, cephalic index, and femur length measurements: not reliable screening techniques for Down syndrome. Obstet Gynecol. 1990 Feb;75(2):186-8.
11. Benacerraf BR, Cnann A, Gelman R, Laboda LA, Frigoletto FD Jr. Can sonographers reliably identify anatomic features associated with Down syndrome in fetuses? Radiology. 1989 Nov;173(2):377-80.
12. Brumfield CG, Hauth JC, Cloud GA, Davis RO, Henson BV, Cosper P. Sonographic measurements and ratios in fetuses with Down syndrome. Obstet Gynecol. 1989 Apr;73(4):644-6.
13. Nyberg DA, Resta RG, Luthy DA, Hickok DE, Williams MA. Humerus and femur length shortening in the detection of Down's syndrome. Am J Obstet Gynecol. 1993 Feb;168(2):534-8.
14. Hill LM, Guzick D, Belfar HL, Hixson J, Rivello D, Rusnak J. The current role of sonography in the detection of Down syndrome. Obstet Gynecol. 1989 Oct;74(4):620-3.
15. Weisz B, David AL, Chitty L, Peebles D, Pandya P, Patel P, Rodeck CH.Association of isolated short femur in the mid-trimester fetus with perinatal outcome. Ultrasound Obstet Gynecol. 2008 May;31(5):512-6. doi: 10.1002/uog.5349.
16. Biagiotti R, Periti E, Cariati E. Humerus and femur length in fetuses with Down syndrome. Prenat Diagn. 1994 Jun;14(6):429-34.
17. Nyberg DA, Resta RG, Hickok DE, Hollenbach KA, Luthy DA, Mahony BS.Femur length shortening in the detection of Down syndrome: is prenatal screening feasible? Am J Obstet Gynecol. 1990 May;162(5):1247-52.
18. Benacerraf BR, Neuberg D, Frigoletto FD Jr. Humeral shortening in second-trimester fetuses with Down syndrome. Obstet Gynecol. 1991 Feb;77(2):223-7.
19. Cuckle H, Wald N, Quinn J, Royston P, Butler L.Ultrasound fetal femur length measurement in the screening for Down's syndrome. Br J Obstet Gynaecol. 1989 Dec;96(12):1373-8.
20. Johnson MP, Barr M Jr, Treadwell MC, Michaelson J, Isada NB, Pryde PG, Dombrowski MP, Cotton DB, Evans MI.Fetal leg and femur/foot length ratio: a marker for trisomy 21. Am J Obstet Gynecol. 1993 Sep;169(3):557-63.
21. Lockwood CJ, Lynch L, Ghidini A, Lapinski R, Berkowitz G, Thayer B, Miller WA.The effect of fetal gender on the prediction of Down syndrome by means of maternal serum alpha-fetoprotein and ultrasonographic parameters. Am J Obstet Gynecol. 1993 Nov;169(5):1190-7.
22. LaFollette L, Filly RA, Anderson R, Golbus MS. Fetal femur length to detect trisomy 21. A reappraisal. J Ultrasound Med. 1989 Dec;8(12):657-60.

23. Lockwood C, Benacerraf B, Krinsky A, Blakemore K, Belanger K, Mahoney M, Hobbins J. A sonographic screening method for Down syndrome. Am J Obstet Gynecol. 1987 Oct;157(4 Pt 1):803-8.
24. Grandjean H, Sarramon MF. Femur/foot length ratio for detection of Down syndrome: results of a multicenter prospective study. The Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant Study Group. Am J Obstet Gynecol. 1995 Jul;173(1):16-9.
25. Dicke JM, Gray DL, Songster GS, Crane JP. Fetal biometry as a screening tool for the detection of chromosomally abnormal pregnancies. Obstet Gynecol. 1989 Nov;74(5):726-9.
26. Johnson MP, Michaelson JE, Barr M Jr, Treadwell MC, Hume RF Jr, Dombrowski MP, Evans MI. Combining humerus and femur length for improved ultrasonographic identification of pregnancies at increased risk for trisomy 21.Am J Obstet Gynecol. 1995 Apr;172(4 Pt 1):1229-35.
27. Viora E, Errante G, Bastonero S, Sciarrone A, Campogrande M. Minor sonographic signs of trisomy 21 at 15-20 weeks' gestation in fetuses born without malformations: a prospective study. Prenat Diagn. 2001 Dec;21(13):1163-6.
28. Bottalico JN, Chen X, Tartaglia M, Rosario B, Yarabothu D, Nelson L. Second-trimester genetic sonogram for detection of fetal chromosomal abnormalities in a community-based antenatal testing unit. Ultrasound Obstet Gynecol. 2009 Feb;33(2):161-8. doi: 10.1002/uog.6220.
29. Vintzileos AM, Egan JF, Smulian JC, Campbell WA, Guzman ER, Rodis JF. Adjusting the risk for trisomy 21 by a simple ultrasound method using fetal long-bone biometry. Obstet Gynecol. 1996 Jun;87(6):953-8.
30. Nyberg DA, Luthy DA, Cheng EY, Sheley RC, Resta RG, Williams MA. Role of prenatal ultrasonography in women with positive screen for Down syndrome on the basis of maternal serum markers. Am J Obstet Gynecol. 1995 Oct;173(4):1030-5.
31. Bahado-Singh RO, Mendilcioglu I, Rowther M, Choi SJ, Oz U, Yousefi NF, Mahoney MJ. Early genetic sonogram for Down syndrome detection. Am J Obstet Gynecol. 2002 Nov;187(5):1235-8.
32. Verdin SM, Economides DL. The role of ultrasonographic markers for trisomy 21 in women with positive serum biochemistry. Br J Obstet Gynaecol. 1998 Jan;105(1):63-7.
33. Schluter PJ, Pritchard G. Mid trimester sonographic findings for the prediction of Down syndrome in a sonographically screened population. Am J Obstet Gynecol. 2005 Jan;192(1):10-6.
34. Marquette GP, Boucher M, Desrochers M, Dallaire L. Screening for trisomy 21 with ultrasonographic determination of biparietal diameter/femurlength ratio. Am J Obstet Gynecol. 1990 Nov;163(5 Pt 1):1604-5.
35. Grist TM, Fuller RW, Albiez KL, Bowie JD. Femur length in the US prediction of trisomy 21 and other chromosomal abnormalities. Radiology. 1990 Mar;174(3 Pt 1):837-9.
36. Benacerraf BR, Neuberg D, Bromley B, Frigoletto FD Jr. Sonographic scoring index for prenatal detection of chromosomal abnormalities. J Ultrasound Med. 1992 Sep;11(9):449-58.
37. Ginsberg N, Cadkin A, Pergament E, Verlinsky Y. Ultrasonographic detection of the second-trimester fetus with trisomy 18 and trisomy 21. Am J Obstet Gynecol. 1990 Oct;163(4 Pt 1):1186-90.
38. Benacerraf BR, Nadel A, Bromley B. Identification of second-trimester fetuses with autosomal trisomy by use of a sonographic scoring index. Radiology. 1994 Oct;193(1):135-40.
39. Sohl BD, Scioscia AL, Budorick NE, Moore TR. Utility of minor ultrasonographic markers in the prediction of abnormal fetal karyotype at a prenatal diagnostic center. Am J Obstet Gynecol. 1999 Oct;181(4):898-903.

40. Wax JR, Cartin A, Pinette MG, Blackstone J. Does the frequency of soft sonographic aneuploidy markers vary by fetal sex? J Ultrasound Med. 2005 Aug;24(8):1059-63.
41. Campbell WA, Vintzileos AM, Rodis JF, Ciarleglio L, Craffey A.Efficacy of the biparietal diameter/femur length ratio to detect Down syndrome in patients with an abnormal biochemical screen.Fetal Diagn Ther. 1994 May-Jun;9(3):175-82.
42. Rodis JF, Vintzileos AM, Fleming AD, Ciarleglio L, Nardi DA, Feeney L, Scorza WE, Campbell WA, Ingardia C. Comparison of humerus length with femur length in fetuses with Down syndrome. Am J Obstet Gynecol. 1991 Oct;165(4 Pt 1):1051-6.
43. Ultrasound detection of Down syndrome: is it really possible?Lynch L, Berkowitz GS, Chitkara U, Wilkins IA, Mehalek KE, Berkowitz RL. Obstet Gynecol. 1989 Feb;73(2):267-70
44. Aagaard-Tillery KM, Malone FD, Nyberg DA, Porter TF, Cuckle HS, Fuchs K, Sullivan L, Comstock CH, Saade GR, Eddleman K, Gross S, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Bianchi DW, D'Alton ME; First and Second Trimester Evaluation of Risk Research Consortium. Role of second-trimester genetic sonography after Down syndrome screening. Obstet Gynecol 2009; 114: 1189–1196.
45. Vergani P, Ghidini A, Weiner S, Locatelli A, Pozzi E, Biffi A. Risk assessment for Down syndrome with genetic sonogram in women at risk. Prenat Diagn 2008; 28: 1144–1148.
46. Bromley B, Lieberman E, Benacerraf BR. The incorporation of maternal age into the sonographic scoring index for the detection at 14-20 weeks of fetuses with Down's syndrome. Ultrasound Obstet Gynecol. 1997 Nov;10(5):321-4.
47. Nyberg DA, Resta RG, Hickok DE, Hollenbach KA, Luthy DA, Mahony BS. Femur length shortening in the detection of Down syndrome: is prenatal screening feasible? Am J Obstet Gynecol. 1990 May;162(5):1247-52.
48. 48.Smith-Bindman R[1], Hosmer W, Feldstein VA, Deeks JJ, Goldberg JD. Second-trimester ultrasound to detect fetuses with Down syndrome: a meta-analysis. JAMA. 2001 Feb 28;285(8):1044-55.
49. Morales-Roselló J(1), Peralta Llorens N. Outcome of fetuses with diagnosis of isolated short femur in the second half of pregnancy. Obstet Gynecol. 2012;2012:268218. doi: 10.5402/2012/268218. Epub 2012 Apr 17.
50. Papageorghiou AT(1), Fratelli N, Leslie K, Bhide A, Thilaganathan B. Outcome of fetuses with antenatally diagnosed short femur. Ultrasound Obstet Gynecol. 2008 May;31(5):507-11. doi: 10.1002/uog.5265.
51. Chitty LS(1), Griffin DR, Meaney C, Barrett A, Khalil A, Pajkrt E, Cole TJ. New aids for the non-invasive prenatal diagnosis of achondroplasia: dysmorphic features, charts of fetal size and molecular confirmation using cell-free fetal DNA in maternal plasma. Ultrasound Obstet Gynecol. 2011 Mar;37(3):283-9. doi: 10.1002/uog.8893. Epub 2011 Feb 1.
52. Arahori H(1), Tamura A, Wasada K, Shimoya K, Wada K, Murata Y, Ozono K. Sonographic femur length to trunk cross area ratio: prediction of fetal outcome in 30 cases in which micromelia was suspected.
53. Kurtz AB, Filly RA, Wapner RJ, Golbus MS, Rifkin MR, Callen PW, Pasto ME. In utero analysis of heterozygous achondroplasia: variable time of onset as detected by femur length measurements. J Ultrasound Med. 1986 Mar;5(3):137-40.
54. Goncalves L, Jeanty P. Fetal biometry of skeletal dysplasias: a multicentric study. J Ultrasound Med. 1994 Dec;13(12):977-85.
55. Mailath-Pokorny M[1], Polterauer S[2], Worda K[1], Springer S[1], Bettelheim D[1]. Isolated Short Fetal Femur Length in the Second Trimester and the Association with Adverse Perinatal Outcome: Experiences from a Tertiary Referral Center. PLoS One. 2015 Jun 5;10(6):e0128820. doi: 10.1371/journal.pone.0128820. eCollection 2015.

56. Aviram A, Bardin R, Wiznitzer A, Yogev Y, Hadar E. Midtrimester Isolated Short Femur Length as a Predictor of Adverse Pregnancy Outcome. Fetal Diagn Ther. 2015;38(3):205-11. doi: 10.1159/000375446. Epub 2015 Mar 12.
57. Goetzinger KR, Cahill AG, Macones GA, Odibo AO. Isolated short femur length on second-trimester sonography: a marker for fetal growth restriction and other adverse perinatal outcomes. J Ultrasound Med. 2012 Dec;31(12):1935-41.
58. Ventura W, Huaman J, Nazario CE, Ingar J, Huertas E, Antonio Limay O. J Clin Ultrasound. 2012 Feb;40(2):63-7. doi: 10.1002/jcu.20889. Epub 2011 Oct 27.Perinatal outcomes after sonographic detection of isolated short femur in the second trimester.
59. Weisz B, David AL, Chitty L, Peebles D, Pandya P, Patel P, Rodeck CH. Association of isolated short femur in the mid-trimester fetus with perinatal outcome. Ultrasound Obstet Gynecol. 2008 May;31(5):512-6. doi: 10.1002/uog.5349.
60. Kaijomaa M, Ulander VM, Ryynanen M, Stefanovic V. Risk of Adverse Outcomes in Euploid Pregnancies With Isolated Short Fetal Femur and Humerus on Second-Trimester Sonography.. J Ultrasound Med. 2016 Dec;35(12):2675-2680.
61. D'Ambrosio V, Pasquali G, Squarcella A. et al. Antenatal diagnosis of proximal focal femoral deficiency: literature review of prenatal sonographic findings. J Clin Ultrasound. Publish Online: 26 September 2015; DOI: 10.1002/jcu.22306
62. Todros T, Massarenti I, Gaglioti P, et al. Fetal short femur length in the second trimester and the outcome of pregnancy. An Int J Of Obstet and Gynaecol. 2004; 111:83–85
63. SOGC. Clinical practice guidelines et al Soft Markers in Obstetric Ultrasound. J Obstet Gynaecol Can. 2005; 27(6):592–612
64. Offiah AC. Skeletal Dysplasias: An Overview. Endocr Dev. 2015;28:259-76. doi: 10.1159/000381051. Epub 2015 Jun 12. Review.